ANNUAL REPORT

NATIONAL INSTITUTE ON AGING

ANNUAL REPORT

July 1, 1975 through June 30, 1976

TABLE OF CONTENTS

NIA Annual Report
July 1, 1975 through June 30, 1976

EXTRAMURAL PROGRAM

In fiscal year 1976 the extramural program continued the activities responding to its Congressional mandate to support research on the biomedical, behavioral, and social aspects of aging. Research grant expenditures increased from $7,753,000 to $9,683,000. The increase was expended mainly in funding non-competing renewals that were not represented in the 1975 budget because they had received two-year funding in 1974. The relation between renewal and new grants in 1976 is shown in the following rable.

Renewal and New Grants Funded in Fiscal Year 1976*

Type of Grant	Number	Funds
Non-Competing Renewal	85	$7,672,412
Competing Renewal or Supplemental	17	1,203,240
New	9	470,220
	111	$9,345,872

Thus only 5% of the research grant budget was spent on completely new projects. Fortunately the 1977 budget contains funds that will permit a substantial number of new projects to be funded.

The grant mechanisms used by the Institute to support research in fiscal year 1976 were with one exception, the project research grant (Code R01) and the program project research grant (Code P01). About 46% of the money supporting research grants went to P01's and about 51% to R01's. The single core support center grant (Code P30) required less than 3% of the research funds.

The extramural staff, while in NICHD and now in NIA, has been concerned about the large fraction of the research funds in large grants such as program projects and center grants. Efforts made in the last 3 years have reduced this fraction from 62% to the current 49%. This year only 22% of discretionary money (that going into Type 1 and Type 2 grants) went into large grants. Additional efforts will be made to at least reach the overall NIH figure of 38%. However, at the same time, the Institute is considering ways to create more core support center grants and specialized research center grants (Code P50). It may prove desirable to convert some P01's to P30-P01 combinations and some to P50's. The expansion of P30-P01 combinations when desirable can be accomplished by the addition of R01's which will be eligible for support services from existing P30's. The expansion in the research budget expected in 1977 will permit the funding of R01's which will improve the balance between large and project grants. In an effort to facilitate the transition of young scientists from a training to an independent status, the Institute has initiated a special research grant program (Code R23) designed for scientists who have recently finished their training and have not received previous research grant support from NIH.

*These figures are approximate since several additional grants were funded in the closing days of fiscal year 1976.

In 1976 the contract program changed to $842, 441 from the 1975 level of $800,000. Contracts were used primarily to provide resources for scientists supported by grants.

NIA's program is heavily concentrated in the biology of aging both at the level of the organ systems and at the level of cellular and molecular function. About 81% of the grant and contract programs support biologic research, about 2% medical research, about 10% psychologic research and about 7% social research.

The budget for manpower development decreased from $1,817,000 in 1975 to $1,762,000 in 1976. The phase-out of the old type training grants (Code T01) and the development of a new program of training based on the National Research Service Awards (Codes F32 and T32) continued. The new program is composed of a mixture of individual postdoctoral awards and institutional pre- and postdoctoral awards. The training program as a whole supported about 96 predoctoral trainees and 50 postdoctoral trainees. These are about evenly divided between trainees in the biological sciences and trainees in the behavioral and social sciences. Part of the training deals with clinical medicine since it is concerned with senile dementia.

The Congressional Act leading to the creation of NIA stated that the Institute would support research and training in the biomedical, behavioral, and social aspects of aging. NIA's current research and training efforts clearly are not evenly distributed among those areas. Most of NIA's current program was transferred to it from NICHD. However, this does not account directly for the current programmatic imbalance since the mission of NICHD in aging research was essentially the same as the mission that NIA now has. However, there are a number of factors of some importance that do explain the imbalance. Aging research whether in NICHD or NIA overlaps with the mission of a number of other Institutes. In the past, proposals dealing with some specific disease process in the elderly have tended to be assigned to the Institute responsible for that disease or for the organ in which the disease occurred. Only now is there beginning to be much interest in medical schools in the special medical problems of the elderly. These two factors were of major importance in excluding much medical research involving patients from the program that NIA now has. The small representation of social sciences is more difficult to explain. Until the last two years social science applications did about as well in the peer review process as did proposals in the biological sciences. However, a great deal fewer investigator-initiated proposals were received in the social than in the biological sciences. Recently, proposals in the social sciences have done poorly in peer review. Perhaps this represents the influx of poor proposals attracted by the creation of the new Institute. Perhaps it indicates a need for peer review that comprehends better the disciplines that constitute the social sciences. At any rate, it is the intent of the Institute to create programs that adequately respond to its Congressional mandate.

The NIA relies heavily on conferences and workshops to stimulate and help investigators coordinate their work in fields of importance to the Institute. The following workshops and conferences were supported in 1976.

Workshop on Cellular Aging

Workshop on Cell Culture in Studies of Senescence

Workshop on Somatic Cell Crosses and Senescence

Workshop on Selection and Development of an F_1 Hybrid for Research on Aging

Workshop on Studies of the Immune System in the Aging Rat

Immunology Workshop for Grantees

Workshop on Differential Life Expectancy

It is clear that the NIA will have to support many more investigators if it is to carry out satisfactorily the mission that Congress has given it. The question, of course, is where these investigators will come from. There are several possible sources. Investigators in other fields may shift to aging research. Newly trained scientists may take postdoctoral training that launches them in research on aging. Predoctoral students can conduct their research in an area of their discipline that bears on aging processes or the problems of the elderly. NIA plans to recruit scientists from all these sources.

The following sections contain discussion of some of the substantive areas in which NIA has or plans to develop programs.

THE USE OF EXPERIMENTAL ANIMALS IN AGING RESEARCH

Understanding of most of the aspects of the biology of man has been based to a large extent on studies of the biology of other living creatures. Probably an understanding of the biology of aging in man will provide no exception to this general rule. There are a number of advantages to the study of aging in other species. The study can be compressed into a short time by using species with a short lifespan. Experimental procedures can be undertaken that are not permissible in man. Particular species may, through particular circumstances, highlight particular aspects of aging.

During the past seven years the extramural program in aging, first in NICHD and now in NIA, has made a major effort to develop sources of suitable animals for scientists who wish to study aging. The major direction has been the selection of mammalian species since their biological processes are similar to those of humans. Most consideration has been given to the use of rats and mice since these are well studied animals widely used by investigators in many fields They are particularly useful in aging research since they are small and thus relatively inexpensive to raise to old age. The word "relatively" should be emphasized since they are not cheap. However, larger mammals are an order of magnitude more expensive than rodents. The difference in lifespans also imposes severe handicaps on scientists studying large mammals. Mice and rats live at the most five years and many strains have a maximum lifespan of three years. Rabbits and dogs may live 15 years and non-human primates 30 or more years. The difficulty in rapidly gathering information by the study of such

larger mammals is obvious. Though rodents offer substantial advantages, the feasibility and possible profitability of studying larger mammals is currently being evaluated again.

When the extramural aging program began to develop rodent colonies 7 years ago it insisted on one particular point -- that was that the animals come from Caesarean-derived stock and that they be maintained behind barriers that reduced the incidence of infection with microbial agents. The animals were not germ-free since they were exposed to a mixture of harmless bacteria to colonize their skin, gastrointestinal tract, and other body regions. The major reason for the barrier was that rats are particularly prone to develop severe chronic respiratory disease that greatly reduces their value as experimental animals by the time they can be considered old. The incidence of this disease is very high in conventional clean animal facilities that raise rats to old age. There was some resistance to this method of breeding animals since it was maintained that it was not natural. There are still those who maintain that barrier-reared animals are less suitable for aging research than animals reared in conventional clean quarters. However, extramural staff still believes that for rats and probably for mice a barrier is desirable. If infection with pathogenic organisms is desired, they can always be introduced under controlled experimental conditions.

The aging program used the contract mechanism to establish colonies of aging rats and mice at the Charles River Laboratories. These animals are from Caesarean-derived stock and are maintained behind barriers. They are well-defined genetically and with respect to their bacterial flora. Sample animals are autopsied and histological sections examined so that they are also well defined pathologically. Their life-tables have been established on the basis of spontaneous mortality in the colonies. These colonies are probably the finest source of aged rodents and matching young controls in the world. They can be purchased by investigators involved in aging research. The funds, of course, usually come from research grants. There was considerable opposition to the establishment of these colonies of old animals when the program was undertaken because it was maintained that old animals could not be shipped across the country. This fear has proven groundless. Old rodents can be flown from Massachusetts to California without excessive mortality. Over the past 18 months more than 80 investigators have acquired aged animals from these colonies to initiate pilot studies, develop techniques, and complete ongoing research on aging.

Surveys are now being undertaken to determine what animals larger than rodents should be aged in colonies. The results are not in yet but it seems probable that animals about the size of dogs with whom certain types of physiological work that is technically difficult in smaller animals may be desirable. Such animals would, for example, facilitate the study of changes in cardiac physiology as a function of age. In addition it may be that for some studies subhuman primates may prove especially valuable because of their close relationship to humans.

Also, a major need is for animals with lifespans shorter than three years. Unfortunately there are no known mammalian species with well-authenticated lifespans that are shorter than three years. Some shrews have been suggested

as possibilities, but what little information NIA has indicates that when shrews are bred under hygienic conditions their lifespans approximate those of mice.

The only animals definitely known to be shorter-lived than rodents are some invertebrates. Many invertebrates have lifespans measured in days. Since many of the cellular processes occurring in metazoa of all degrees of complexity are similar, it appears possible that important metazoan aging processes at a cellular level may be similar. For this reason investigators have sought invertebrate models for human aging. Several different invertebrates have been studied. Some particularly rewarding results have been found using a roundworm (nematode) with a lifespan of about 30 days. In these animals a progressive loss of enzymatic activity per unit weight of enzyme has been found with increasing age. A search for a similar process is now underway in mammals. If this succeeds, it will present an example of how exploratory work in lower life forms can accelerate our understanding of mammalian aging.

CELLULAR AGING

In the process of evolution, it is generally assumed that multicellular organisms emerged from unicellular life forms which probably did not manifest senescence as we know it. The complexities of the multicellular animals included the development of two major types of cells: the sexual cells (germ line) and the somatic (body) cells. These species were maintained by the periodic fusion of sexual cells which created new organisms composed of both sexual and somatic cells. These organisms carried the sexual cells until the reproductive process was repeated, a cycle essential for the maintenance of the species. There was no necessity for the somatic cells of the individual organism to survive indefinitely; thus, selective forces led to relatively well-defined individual lifespans compatible with the continued existence of populations.

The cells of metazoans can be classified as those that have lost the ability to divide (fixed post-mitotic cells) or as dividing cells. There are no known fixed post-mitotic cells with an indefinite lifespan. The situation is different for cell lines that continue to divide within the individual. The potential immortality of some types of somatic cells has persisted in some animals and disappeared in others. The persistence of potentially immortal cells is striking in some lower invertebrates--flatworms, for example. This species can be propagated indefinitely simply by cutting individual flatworms in two.

In the course of evolution, mammalian body cells, having no need for this potential immortality, may have lost it. At least no normal mammalian body cell type has been discovered that is capable of indefinite propagation. This limitation on lifespan is not a necessary characteristic of mammalian cells. Many types of mammalian cancer cells are potentially immortal, and can be transferred from host to host indefinitely. Some mammalian cells that have undergone certain transformations in tissue culture can also be grown indefinitely in such culture.

It appears probable that normal mammalian body cells age and die because they

cannot repair damage sustained from their own metabolic processes or from adverse environmental effects. Certainly the development and genetic transfer of repair processes for the key deteriorative processes involved in senescence would have imposed a heavy genetic burden on any complex species. A number of investigators, some of them Institute-supported, have made progress in recent years in the development of methods for studying several lines of replicating normal cells. All these lines have a limited ability to propagate themselves. The normal human fibroblast can be grown in tissue culture. Investigations are more advanced in the study of human fibroblast-like cells than in other systems.

Research on cellular aging is being encouraged on cell types other than the fibroblast-like cell, and through the use of techniques which enable "genetic dissection" of somatic cells. To facilitate the growth of such research, the NIA supports a cell bank at the Institute for Medical Research (Murphy, D.G. and W.W. Nichols, Cytogenet. Cell Genet. 15, 30-40, 1975). This bank provides a service to grantees and potential grantees by collaborating in the establishment of genetically marked and tissue-specific cell lines. It also provides related services, such as karyology and limited characterization of the cell lines. The contract supports workshops to encourage concept and experimental design development in the area of somatic cell genetics as applied to aging research.

The NIA conducted a study inviting expert opinion on existing policy to encourage studies of cell lineage in vitro and if feasible in vivo. This study resulted in a strong endorsement of NIA supporting (through the grant mechanism) studies of cell lineages to provide knowledge of lineage patterns and phenomena which contribute to differentiation and aging. This report has been published (Murphy, D.G., Mechanisms of Aging and Development 4(5-6), 317-324, 1975).

IMMUNOLOGICAL AGING

In man, as well as in mice, there is good evidence of a reciprocal increase in certain autoimmune phenomena and malignancies paralleling the decline in immune vigor. The observations by Thomsen in 1929 showed that the humoral immune response declined with aging in man. It is now well-established that the functions of the cellular immune system also decline markedly with age.

Immune function over the lifespan of the individual increases rapidly in the first years of life, reaches a peak in the teens, and then declines progressively. It is reported that an elderly person has about one-tenth the immune competence of a teenager. Several major changes take place as the immune system ages. There is a loss of antibody-forming cell precursors from the peripheral lymphoid organs, changes of the architecture of the peripheral lymphoid tissue, and a generalized loss of thymus-derived cell function. Relatively early in life, atrophy of the thymus begins and slowly progresses with age.

Since immunologically incompetent individuals are very susceptible to infections, this probably is one of the major sources of their health problems. Several studies in animals have shown that manipulation of the immune system

by experimental means results in an increased lifespan. Institute-supported studies have shown that dietary restriction profoundly affects the immune system of mice. These mice show anatomic and certain immune functional changes which suggest the immune system may mature less rapidly. It also appears to age more slowly after maturation. Furthermore, dietary restriction results in prolongation of the lifespan. The possibility that immune competence can be improved in an incompetent individual makes this area of research particularly important in terms of the potential for application to humans.

This Institute has the sound beginnings of a program on the effects of aging on the immune system and intends to develop it further.

MENOPAUSE

The physical symptoms of menopause reflect changes in the aging ovary which are of considerable interest to research on aging. Much speculation has centered on whether decline in endocrine function and in particular in production of sex hormones plays a major role in the aging process. This speculation has been largely concerned with the aging ovary and the ensuing menopause (cessation of menses) that occurs at approximately age 50. Ovarian involution, atresia and cessation of estrogen production remain important objects for research on aging, particularly in view of recent controversy about the efficacy of current estrogen replacement therapy.

The ovary's age-associated changes in functional production of estrogen and in morphology are more pronounced than changes in any other body organ. The changes from quiescence of childhood to active formation of estrogen-producing follicles at puberty and subsequent decline in estrogen production and involution of the ovary at about age 50 represent remarkable changes in the morphology and function of this organ. The latter changes are important because of their relationship to age-associated degenerative changes that occur at or near the onset of menopause. The decline and virtual cessation of ovarian production of estrogens cause hot flushes and genital atrophy. A relation of estrogen deficiency to osteoporosis, arteriosclerosis, mucous membrane deterioration, disorders of the skin and genitourinary system and possibly psychological impairment has been suspected but not proven.

Although some of the immediate effects of decline in ovarian function such as cessation of menses, hot flushes and genital atrophy are known, the relationship of the menopause to many other aspects of aging and disease has not been extensively studied. Clearly, the menopause is much more complex than simply a decline or cessation of estrogen production which can be compensated for by the administration of estrogens. The triggering mechanism for the disappearance of the ovarian follicle and decline in estrogen production by about age 50 is largely unknown. There is a lack of hard data on metabolic changes that can be attributed to the decrease of sex steroid in menopausal women. Even less information is available on the effects of long-term administration of sex steroids to postmenopausal women to compensate for sex hormone imbalance.

In aging women, the value of prophylactic administration of estrogens to prevent coronary artery disease, osteoporosis or changes other than genital atrophy and hot flushes has not been conclusively demonstrated. While several

studies suggest that estrogen prophylaxis may be beneficial, there have been no definitive studies on the subject. In view of firm data on increased thromboembolic disease in women on oral contraceptives, the risks and benefits of replacement therapy need careful examination, particularly since it is estimated that 13% of postmenopausal women are currently taking some type of intermittent replacement therapy.

The menopause, its sequelae and treatment have major public health implications since they affect the health and well-being of a major segment of the population. Studies on the mechanism triggering ovarian involution, estrogen sources and production, as well as studies on the risks and benefits of different sex steroids are needed to understand the menopause and its relationship to aging processes and degenerative diseases.

The extramural program supports research on the mechanisms aimed at acquiring information and data on understanding the mechanisms involved in ovarian involution. Also, to a lesser extent studies are currently supported to determine whether the relative risk of adverse side effects are greater in women on estrogenic compounds.

COGNITIVE CHANGE WITH AGE

Studies of cognitive and intellectual changes with age and the morphological, physiological, and biochemical changes in the central nervous system which probably underlie these changes are of major concern to the Institute. From such studies may flow information to modify these changes and an understanding of the structuring of society that will best adjust to them.

There are major changes in mental function across the years. Many of these changes are easily discernable by observation. Such observations have made us aware of the speed with which children learn and forget new languages in contrast to adults, the importance of early entry into many types of activity in determining later competence, the greater creativity of the years of late adolescence and early adulthood, the improve judgment that goes with the later years, and the senile dementias. These all attest to changes in mental function with age. However, this knowledge is not the result of controlled experimentation and may be misleading. Uncontrolled and even unrecognized variables may be operating that make much of what we think we know about this subject unreliable.

Most studies of intellectual competence as a function of age have been cross-sectional, that is, they have compared the function of individuals of different ages at the same point in time. These studies have shown a decline in intellectual capacities with age. The declines in general have been small with tests of verbal ability and larger with tests of problem-solving ability. Institute-supported studies have cast doubt on these findings. When individuals are tested at two points in time, making possible the measurement of change in each of many individuals, there may be little or no decline in measures of intelligence before the onset of extreme old age.

However, this generally benign change in intellectual competence is not universal. Institute-supported studies have shown that intelligence declines more

rapidly in the later years of life in persons with high blood pressure than in those with normal blood pressure. Mental competence can be reduced in the later years of life by depression and fortunately this reduction can be reversed by successful treatment of the depression. In addition about 5% of persons over the age of 65 years have neuronal degeneration that produces severe dementia. This neuronal degeneration is probably the most common cause of what is called senile dementia.

The Institute considers research on senile dementia to be one of its top priorities. With increased control of atherosclerosis and cancer and with more persons surviving into their 70's and 80's, this may become one of the most common diseases in this country. The Institute now supports two training programs in senile dementia. Institute-supported investigators have made important discoveries that provide leads for future research. They have shown that there is an appreciable decline in learning ability in rats as they age. Rats thus provide an experimental mammalian model for the study of cognitive change with age. They have also shown a progressive loss of connections between the brain cells of aging rats. This suggests at least one mechanism for a loss of cognitive ability with age.

SOCIETAL ASPECTS OF AGING

The average duration of life has changed greatly since the evolutionary emergence of mankind, and there have been corresponding changes in the age structures of the many different populations that have existed on the earth. There is no reason to think that these age-structures will not continue to change and it is possible that they will be exaggerated by major changes in the average length of life. The human race has adjusted to a great variety of age structures successfully in the past and will probably continue to do so in the future.

Future changes in mortality rates will probably play a minor role in determining total population. For example, doubling the average lifespan would only double the population and would require an average human lifespan to do so. Fertility is the major cause of explosion and on a global scale is doubling the population every 30 years.

Fertility rates are also very important in determining what fraction of the population is elderly. Ten percent of the population is now over 65 years of age. With zero population growth, current mortality rates, and no migration this country will ultimately have 16% of its people over that age. Decreased mortality rates due to control of vascular disease could increase the percentage of the elderly even more and create problems if the increase in lifespan were not accompanied by a substantial decrease in the deteriorative processes of aging. For this reason the Institute supports research to investigate the implications of changes in mortality rates for society and the individuals who comprise it.

THE FUTURE

The extramural program will have to be broadened considerably to fulfill the total mission given it by Congress. There are a number of serious gaps of

which geriatric medicine and societal aspects of aging are probably the largest. In the coming year a great deal of effort must be devoted to the delineation of the Institute's program in what it should support and what it should leave to other agencies.

NIA Annual Report
July 1, 1975 through June 30, 1976

Adult Development and Aging Branch
Contract and Collaborative Research

Contract Number: AG-3-2725

Contract Title: Contract to Breed, Rear and Maintain a Colony of Inbred Aging Laboratory Rats for Aging Research (Modified)

Contractor: Charles River Breeding Laboratories, Wilmington, Massachusetts

Money Allocated: $170,000 FY 1976

Objectives:

1. Meet current and projected demands for senescent laboratory rats reared on a defined diet in a specific pathogen-free environment.

2. Establish a standing commercial resource of senescent rats on which investigators can immediately draw for aged laboratory rats.

3. Develop baseline physiological and pathological characterization of the Fischer 344 rat over its full lifespan.

4. Establish survival curves for laboratory rats reared specific pathogen-free behind a defined barrier system.

5. Increase the numbers and ages of animals to be made available for studies in aging.

Significance for Aging Research: A major constraint influencing the development of aging research has been the almost total absence of an aged animal resource sufficiently characterized to meet the unique needs of aging research. The development of a colony of aging laboratory rats under this contract will significantly enhance the quality and quantity of aging research by providing aged animals that are reared in a defined environment on a standardized diet, free of pathogenic organisms, and characterized with regard to age-specific causes of death.

Basic to the development of studies in aging research in animals is a characterization of expected physiological and pathological changes that may occur over the animal's full lifespan as well as life tables that accurately reflect survival at specific ages. A primary aim of this contract is to acquire this data and make it available to investigators in aging. With this information, a reasonable comparative assessment can be made as to whether the animals, strain or stock is suitable for studies in aging. Also, within reasonable limits, numbers of animals needed for statistical significance of studies can be readily established, thus minimizing the likelihood of supporting excessive numbers of animals or too few animals for statistical significance of the study.

Currently many investigators in aging cannot acquire aged animals short of rearing the animals themselves, nor are they able to maintain aging rats under the laboratory conditions necessary to allow the animals to survive long enough to observe truly senescent change with age. Also, competent young investigators more often than not cannot support aging colonies of rats until they successfully compete for research support. Without this resource many imaginative young investigators will continue to be excluded from research in aging simply because they are unable to identify an aged animal resource which they could use in the studies they propose in aging. Thus far this colony of aged rats has served as a resource for the study of aging in more than 40 individual research projects.

Proposed Course: Contract is to be continued for a minimum of two years with the contract becoming increasingly self-sustaining.

NIA ANNUAL REPORT
July 1, 1975 through June 30, 1976

Adult Development and Aging Branch
Contract and Collaborative Research

Contract Number: AG-6-2136

Contract Title: Aging Barrier Sprague-Dawley Rat Colony

Contractor: Harlan Industries Incorporated, Cumberland, Indiana

Money Allocated: $48,245

Objectives: 1. To meet the current demand for a commonly used stock of laboratory rat.

2. To develop a commercial resource of aged virgin male rats on a defined diet in an essentially pathogen-free environment for investigators in aging research.

3. To provide a ready resource of aged rats that may be used to develop techniques and procedures for studies on aging as well as pilot studies for the acquisition of preliminary data as a basis for determining feasibility for research on aging.

Significance for Aging Research: One of the primary barriers to the development of a program of studies in aging research is the lack of availability of laboratory animals in varying degrees of senescence that are representative of the aging process. To facilitate the development of aging research, standardized strains, stocks and species of animals that can be used as simulation models of aging processes must be developed in quantities that meet the needs of investigators in aging research. Since a single strain, such as the Fischer 344 cannot serve as a model for all studies, a commonly used rat of different genetic characteristics is required for a significant number of studies in aging. The Sprague-Dawley represents a distinctly different rat model for the study of aging.

Proposed Course: The colony will be maintained for a minimum of five years.

NIA ANNUAL REPORT
July 1, 1975 through June 30, 1976

Adult Development and Aging Branch
Contracts and Collaborative Research

Contract Number: AG-6-2135

Contract Title: Selection and Development of an F_1 Hybrid Aging Rat Strain

Contractor: Charles River Breeding Laboratories, Wilmington, Massachusetts

Money Allocated: $93,265

Objectives: 1. Development of a rat model for aging research that provides a broad gene pool and maximum genetic control.

2. Develop an F_1 hybrid strain that avoids pathologic lesions of body systems and endocrine tumors that are seen in most inbred strains and outbred stocks of rats currently available.

3. Provide a strain of rat for research on aging that is less susceptible to environmental change than are in the currently available inbred strains.

4. Provide a rat strain which has much of the generalizability to aging as an outbred stock with uniformity and predictability of pathologic and biologic characteristics of the inbred strains.

5. Characterize the major biologic and pathologic changes over the life span of the strains selected for development.

6. Select from among three between-strains crosses with the Fischer 344 the F_1 hybrid cross representative of the characteristics believed to be of greatest general applicability to the studies of aging in the rat.

Significance for Aging Research: Studies utilizing animal model systems are unique with respect to the considerations that must be exercised in the development of aging animals. Current and projected experiments in aging will require animals of defined genetic background, known biological characteristics and environmental status. Only with meticulous and exacting control of the many interacting genetic, physiologic, pathologic and environmental variables will it be possible to develop relevant animal models that may explain many of the biologic processes in aging. The F_1 hybrid rat model is necessary in aging research in view of the need for a rat model that is genetically defined, characterized biologically and represents a broad gene pool that permits generalizations of findings to the species as a whole.

Proposed Course: Study completed, for selection of F_1 Hybrid January 1979. Development of F_1 Hybrid strain should commence in FY 1980.

NIA Annual Report
July 1, 1975 through June 30, 1976

Adult Development and Aging Branch
Contract and Collaborative Research

Contract Number: AG-5-2854

Contract Title: Aging Monkey Tissues and Organ Resource

Contractor: Washington State University, Pullman, Wash.

Money Allocated: $10,869.00 (FY 1976) continued fron FY 1975

Objectives: 1. To acquire organs and tissues from a rare resource of six (6) aged rhesus monkeys age 24 to 26 years as the animals become moribund or expire.

2. To select and preserve organs and tissues from each of the animals that are or may be required for the study and inter-species comparison of aging and aged changes in the Rhesus monkey and other mammalian species.

3. To bank fresh, frozen or chemically fixed and preserved tissues and organs from each of the six monkeys as they become moribund or expire.

4. To provide selected tissues and organs on request for studies in aging.

Significance for Aging Research: The study of aging requires the availability of tissues and organs from a wide variety of strains and species of animals. To study aging changes and the comparative differences between the ordered lifespan of different species of mammals requires that the program identify and develop resources that meet the needs of the investigator in aging research. Preservation and provision of tissues and organs from aged subhuman primates, essentially expiring from natural causes, will provide a continuing resource of rare and unique materials that would otherwise be lost to aging research. The contract essentially supports the complete postmortem evaluation and preservation of tissues and organs of each of the six monkeys as they become moribund and/or expire. Postmortem protocol will require that all tissues and organs be examined, classified, and characterized. Tissues and organs from all major body systems and the integument will be selectively preserved based primarily on the requirements of the individual investigators. Other tissues and organs will be preserved by freezing or fixed in chemicals as well as preparation of slide sets of tissues from major organ systems. These materials can be provided on request for study of aging changes in the subhuman primate or comparative studies between species.

Proposed Course: Contract is to be continued for a minimum of two years.

NIA ANNUAL REPORT
July 1, 1975 through June 30, 1976

Adult Development and Aging Branch
Contract and Collaborative Research

Contract Number: AG-6-2145

Contract Title: Evaluation of comparability of the Macca Nemestrina as a model of aging in man.

Contractor: University of Washington, Seattle, Washington.

Money Allocated: $20,479 (FY 1976)

Objectives: 1. To evaluate the utility of the non-human primate (M. Nemestrina) model of physical aging.

2. To identify, collect, evaluate and provide organs and tissue for collaborative study and characterization with other investigators.

3. To determine whether the quality and direction of physical changes are comparable to those seen in human aging.

4. To maintain a reserve of ten aged 18-24-year old M. Nemestrina as a ready resource for later study, dependent on determination of their comparability to aging processes in the human.

5. To provide NIA with a review of the literature on aging in non-human primates incorporating the findings from the comparative study of various physiologic systems in the 10-year old and 20-year old (M. Nemestrina) pigtail macca.

Significance for Aging Research: The study of aging requires the characterization and availability of a wide variety of strains and species of animals as well as resource materials from aged animals. To study aging changes and the differences between species of mammals as well as their relevance as models for processes of aging, the NIA must identify, characterize and develop resources that meet the needs of investigators in aging. The characterization and maintenance of an aged resource of non-human primates and the evaluation, preservation and provision of tissues and organs simultaneously retains and provides rare and unique materials that would otherwise be lost to aging research.

Proposed Course: Contract will continue for a minimum of one year and will be continued dependent on the evaluation of the model and the demand for biological materials from this resource.

NIA Annual Report
July 1, 1975 through June 30, 1976

Adult Development and Aging Branch
Contract and Collaborative Research

Contract Number: AG-6-2134

Contract Title: Aging, Estrogen Use, Hypertension and Myocardial Infarction

Contractor: University of California at Irvine, Irvine, California

Money Allocated: $165,000 (FY 1976)

Objectives: The aim of the proposed epidemiologic study is to determine whether and to what extent estrogen usage tends to increase the risk of hypertension and myocardial infarction (MI) in a population of postmenopausal women living in a retirement community. The contract provides for the support of a retrospective study of the incidence of hypertension and MI as well as other factors which predispose to hypertension and MI in a specific and uniquely discrete population of 9,000 postmenopausal women. Cases of hypertension and MI occurring in postmenopausal women will be identified and described (morbidity and mortality). The population of identified hypertension and MI cases will be compared with an appropriate control group to determine if the case group is significantly different from that of the controls. Also, to determine the role of likely risk factors, proximity, dose and duration of drug usage in affecting hypertension and MI.

Significance to Aging Research: Until recently, the long-term effects of estrogens in postmenopausal women have been a matter of conjecture or largely ignored. The proposed study will provide current data on comparative risk of hypertension and MI in postmenopausal women taking estrogens and postmenopausal women who are not using them, but are otherwise at comparable risk from other causes.

The significance of this project lies in the fact that a substantial percentage of the women in the population are taking estrogen-like medications and the initial goal of the study can be completed within a two and one-half year period. At present the value of estrogen-like medication as therapy for post menopausal symptoms, for the prevention of vascular disease, and for the arrest of osteoporosis remains unproven. There is serious concern that such medication in commonly used dosage is a significant health hazard with specific reference to the three most common causes of death--heart disease, cancer, and stroke. There are not data available on the questions posed by this proposal. The population selected is uniquely constructed to permit this type of investigation. Information of this type is extremely important, since it may serve to guide the medical care of the many millions of women over the age of 50 in the United States.

Proposed Course: The study is planned for a minimum of two years.

NIA ANNUAL REPORT
July 1, 1975 through June 30, 1976

Adult Development and Aging Branch
Contract and Collaborative Research

Contract Number: Professional Services Contract

Contract Title: Survey of Former "ADAB" Trainees

Contractor: Dr. Thomas I. Myers, Bethesda, Maryland

Money Allocated: $4,000 FY 1976

Objectives: To contact each scientist who completed training at the Ph.D. or post-doctoral level with the aid of stipends from ADAB Training Grants or Fellowships, and obtain information on the nature of his present employment and whether or not he or she is engaged in research, teaching, or other professional level activities in the field of aging.

Significance for Aging Research: To guage the success of NIA research-training programs, both individually and collectively, in producing scientists specializing in problems of aging or the aged.

Proposed Course: The survey will be completed before June 30, 1976.

Adult Development and Aging Branch
Contract and Collaborative Research

Contract Number: NIH-AG-76-14

Contract Title: A Study of Lymphocyte Function and Serum Immunoglobulin Concentration During the Lifespan of Individual Mice

Contractor: Cornell University, New York, New York
(Contract Officer: Dr. Marc Weksler)

Money Allocated: $42,400

Old animals are immunologically impaired, displaying depressed and at times disordered immune reactivity. Old mice produce fewer plaque forming cells and lower concentrations of serum antibody following immmunization (1-5) and reject skin or tumor grafts more slowly (1-6) than do young animals. In addition, lymphocytes from old mice do not induce a graft versus host reaction (7) or proliferate in culture with phytohemagglutinin (8-9) in a normal fashion.

The impaired immune function of old mice is manifested not only by depressed humoral and cellular immunity but also by the spontaneous appearance of antibodies to autologous cells and tissues. Antibody to syngeneic erythrocytes is found in serum from many aged mice (10). Whether autoimmunity is concordant with impaired immune reactivity is not known. That is, it is not known whether autoantibodies develop in old mice which are most or least immunologically impaired.

Although defects in immune function of aged mice have been repeatedly demonstrated, it has never been shown whether immune reactivity has a positive, negative or little survival value. That is, do animals with vigorous immune reactivity die early while those possessing lower reactivity survive or does immune reactivity decline with age and those with the most impaired immune function die first. Either hypothesis would result in the observed impaired immune function observed in old mice. This contract is directed at these questions.

This contract supports a prospective study to assess immune reactivity, by measuring the proliferative response of blood lymphocytes in culture and by measuring the plasma concentration of immunoglobulins. These immunological parameters will be correlated with the development of autoimmunity and longevity.

NIA Annual Report
July 1, 1975 through June 30, 1976

Adult Development and Aging Branch
Contract and Collaborative Research

Contract Number: AG-2-2755

Contract Title: Quantitative Studies of Aging Human Diploid Fibroblasts *in vitro*

Contractor: University of Vermont
(Contract Officer: Dr. Marlene Absher)

Money Allocated: Continued with no additional funds (FY 1976)

Objectives: The Contractor is describing the division patterns and cellular lineages of human cells grown in culture utilizing time-lapse cinematographic, autoradiographic and computer analysis and model simulation techniques.

Significance for Aging Research: The human diploid cell in culture is a widely-studied model for aging. Populations of these cells double actively under standard cell culture procedures for many months, but eventually age and die. Although extensive research is being conducted on populations of such cells, the studies are being pursued without definitive knowledge of the division charcteristics of individual cells and their progeny. There is evidence that "old" populations contain many "young" behaving cells, just as "young" populations contain "old" behaving cells no longer capable of division. These and other data are being produced by Dr. Absher to the end of refining experimental design, cellular aging research concepts and hypotheses, and a capacity to relate cell culture studies of aging to the aging process in man.

NIA Annual Report
July 1, 1975 through June 30, 1976

Adult Development and Aging Branch
Contract and Collaborative Research

Contract Number: NO1-AG-4-2865

Contract Title: Selection, Production, Characterization and Distribution of Genetically Marked Cells for Aging Research

Contractor: Institute for Medical Research, Camden, New Jersey
(Contract Officer: Dr. Warren W. Nichols)

Money Allocated: $131,237.00 (FY 1976)

Objectives: To establish and maintain a repository of frozen viable genetic and mutant cell cultures that are of interest in aging research and to hold a workshop addressing the use of genetically marked cells for aging research.

Significance to Aging Research: The in vitro expression of genetic uniqueness of different cell strains offers powerful means to investigate mechanisms of aging at the cellular and subcellular level. This contract is to encourage the use of somatic cell genetics in studies of cellular aging; provide characterized, contaminant-free cell cultures to qualified investigators; and enhance research through communicative activities such as the annual workshop. The Institute for Medical Research resource is to be a focal point of grant program activities in cellular aging.

NIA Annual Report
July 1, 1975 through June 30, 1976

Gerontology Research Center

The Intramural Research Program of the NIA is located at the Gerontology Research Center, Baltimore, Maryland. The Center occupies a separate four story building containing approximately 100,000 sq. ft. of laboratory, animal, and office space located on the grounds of the Baltimore City Hospitals. The building is situated on approximately 5 acres of ground which was deeded to the Federal Government by the City of Baltimore in 1965. Construction of the building was completed in 1968.

The program of the GRC is organized under four Laboratories and Branches, viz., Laboratory of Behavioral Sciences, the Clinical Physiology Branch, the Laboratory of Cellular and Comparative Physiology, and the Laboratory of Molecular Aging. The Office of the Chief provides research services (technical development, electronic data processing, photography and arts, animal resources, information services, and library services) to all operating Branches and Laboratories, as well as supervision of the Collaborative Guest Scientist Program.

In FY 1976 46 visiting scientists, visiting fellows, and guest scientists with 27 supporting personnel worked in GRC laboratories in addition to those occupying the 154 budgeted positions assigned to GRC (58 professional, 96 supporting personnel). Another 46 employees in part-time, temporary, WAE, stay-in-school, and work-study categories participated in the programs of the GRC.

No new budgeted positions were assigned to the GRC during FY 1976.

Office of the Chief

Research Services

The Technical Development Section has continued to maintain and service scientific equipment for all investigators at the GRC. Specialized equipment has been designed and constructed for a number of research programs. For example, a high speed filtration chamber for the Molecular Chemistry Section; equipment for the humane sacrifice of rabbits and rats, as well as special metabolism cages for dogs (Animal Resources Facility); a unique device for long term partial occlusion of the aorta in intact rats (Cardiovascular Section), and modifications to Bowman rat holding devices (Laboratory of Cellular and Comparative Physiology).

A digital data acquisition system is currently being developed for the Cardiovascular Section. This system will permit the various projects in this section to collect experimental data in digital form for later analysis on the GRC computer system. A number of laboratory interfaces, including small graphic display terminals, are being designed and fabricated.

In support of these laboratory data acquisition systems, a cassette tape system was designed and installed in the GRC computer system. In addition, all software required for these systems was written by the Technical Development Section.

The Animal Resources Facility provided housing and care for approximately 11,000 rats, 9,000 mice, 12 guinea pigs, 90 rabbits (2,500 rabbits were received and issued), 35 beagles (aged 2-10 years), 8 monkeys, and 2 goats to support research programs at the GRC.

The closed production colony of outbred Wistar-derived rats is being maintained at approximately 10,000 animals. During the year 6,457 weanling rats were added to the colony and 1,484 were issued. Of the rats issued, 687 were 1-11 months old; 267 were 12-23 months old; and 267 were 24 months old or older. To meet changing program needs two additional strains of rats were introduced into the colony. Comparative data on mean life span, weight, and age-related pathology is currently being gathered on these new strains to assess their suitability as models for aging research.

During the year a catastrophic parainfluenza (Sendai virus) infection was introduced into the mouse colony from mice shipped to the GRC from NIH production colonies. This incident resulted in the termination of seven individual research projects which had been following animals for 2 to 3 years. A total of about 2,000 mice died or had to be destroyed. This was a serious blow to long term projects which are essential for the study of aging.

Animal health surveillance has been increased. A quarterly health status report based on bacterial, viral, parasite, and pathological monitoring of all rats and mice in the colony is now being issued.

The Photographic and Arts Section provided 467 drawings, 4,247 photographic prints, and 1,952 slides in support of GRC scientists during the year.

A variety of services were provided by the Information Office during the year. Foreign scientists from Canada, India, Japan, and Poland visited the GRC and conferred with staff. TV documentary crews from Tokyo, Japan and the French language division of the Canadian Broadcasting System shot footage for upcoming programs. Members of the Maryland Association of Science Teachers were briefed on NIA activities, and throughout the year medical, university, nursing, and high school students were briefed and toured. In all, 38 tours and briefings were held for 245 people.

Contacts from the news media resulted in GRC staff appearing on WMAR-TV (Baltimore), WPTV-TV (Florida), and WAMU-FM Educational Radio (Washington). A major story on the NIA and GRC is being prepared by Fortune Magazine for later this year. Articles dealing with Center studies or scientists appeared in U.S. Medicine, BioSciences, the Jerusalem (Israel) Post, the Baltimore Sun, the Washington Star-News, Parade, Aging, the Louisville Times, and the Louisville Courier-Journal.

Professional and specialty media served included: Physicians Radio

Network; JAMA Medical News; Medical Tribune; Dynamic Maturity; Retirement Living; Washington Report on Long-Term Care; Geriatrics; Aging; Journal of Gerontological Nursing; Pharmacy Times; Ageing International, and Aging Tomorrow.

The GRC Library staff indexed 3,961 titles for publication in the bimonthly Journal of Gerontology feature, "Current Publications in Gerontology and Geriatrics." Scientists, students and other publics made increasing use of the library as the regional repository of complete information on subjects dealing with aging.

Collaborative Guest Scientist Program

During the year 12 programs were supported by the GRC. These programs were staffed by 26 investigators with 27 supporting personnel. The investigations were carried out at all levels of tissue organization from cells grown in culture to man.

Cell Biology. Cellular loss has frequently been described as a consequence of senescence. Therefore studies have been carried out to identify factors which influence cellular replication. A loss of proliferative capacities of cultured human diploid fibroblasts (WI-38) either with increased passages or with increased age of the donor has been repeatedly described. In an attempt to identify changes associated with cellular senescence, surface characteristics have been compared in WI-38 cells and in cells of the transformed line VA13A/2A which retain high proliferative capacities. The results indicate that the mitogen ConA-HRP is more randomly distributed on the surface of the transformed cells than that of WI-38. In addition, ConA binding patterns seen in 3T3 (mouse fibroblasts) were unaffected by X-irradiation (400 rads). However, X-irradiation resulted in a decreased binding of ConA to the surface of SV3T3 (transformed) cells. This suggests a greater sensitivity of the surface of transformed cells which maintain replicative capacity.

Regional differences in the regenerative capacity of the earthworm makes this organism a useful model to study factors which control cellular proliferation. Separation of proteins by electrofocusing showed a different pattern for extracts from segments which had no anatomical differences but varied greatly in proliferative capacity. The molecular weight of these proteins which appeared in areas with lowered replicative capacity was about 25,000, which is similar to that of some chalones known to inhibit cellular replication.

The effects of aging on the cellular synthesis of proteins is a key question in gerontology. The levels of microsomal RNA and proteins, cytosol proteins, and free amino acids which play an important role in protein synthesis were essentially the same in the livers of senescent (20-32 mos.) and adult (12 mo.) rats. However, the incorporation of amino acids into proteins of a cell free system isolated from senescent rats was 28% lower than those isolated from adult animals. Substitution of either microsomes, T-RNA fractions or T-RNA free PH5 enzyme fractions isolated from senescent animals for those isolated from young animals decreased protein

synthesis. Therefore, age-related deficiencies seem to occur in many of the components necessary for protein synthesis in cell free systems. In addition, the optimal Mg^{++} concentration for maximal protein synthesis in systems isolated from young animals is greater than that from senescent rats. Even at the optimal level of Mg^{++}, however, the senescent system is less active than the adult system. These findings may suggest age changes in the binding capacities of enzymes or membranes.

Efforts have been made to establish the influence of age on the cytolytic and neuropathic effects of vincristine and vinblastine. In this first series of experiments there were no significant differences in the binding of vinblastine to tubulin, the protein subunits of microtubules, of newborn rats and adult rat brains. Since it is known that young humans can tolerate higher doses of related compounds before developing significant neuropathic changes, these results indicate that the age differences cannot be attributable to the binding properties of tubulin.

Cardiac Performance. The myocardium of the aged rat is characterized by a decrease in the intrinsic contractile ability and an increase in static and dynamic stiffness. In the cardiac muscle of aging rats the ability to develop tension is maintained by the increase in stiffness in either a delayed onset of relaxation or a delay in the time course of relaxation. Studies suggest that the mechanism of the age-associated prolongation of contraction is a delay in calcium uptake by the active relaxing system. Other studies indicate that the aged myocardium has a decreased responsiveness to agents which require a cell membrane receptor. However, the inotropic response to reagents that do not require such receptors are well maintained at advanced ages. In addition, echocardiographic techniques have identified significant age associated changes in left ventricular wall thickness, mitral valve motion, and aortic diameter of men. Finally, an age-dependent decreased response of the sinus node to chronotropic stimulation by catecholamines has been demonstrated in anesthetized dogs.

Treatment of Incontinence. Fecal incontinence is common among the elderly. Forty subjects were treated with operant conditioning (biofeedback training); 28 achieved a good symptomatic response to operant conditioning as evidenced by disappearance of incontinence or by decrease in frequency by greater than 90%. Maximal improvement occurred after one session of operant conditioning in 20 out of the 28 responders. During a follow-up period ranging from 4 months to 8 years, only two patients regressed temporarily. They responded to repeated conditioning indicating that improvement attained by this technique may be sustained for long periods of time, if not permanently.

Learning and Memory. Deficits in learning and memory are commonly associated with aging. The extent of changes in non-cognitive functions attributed to these losses is not well understood. Arousal is one important component of cognitive performance. Earlier studies have indicated that the elderly show lower arousal than younger individuals during some learning tasks. Data from this investigation supports this concept in that elderly subjects showed smaller increases in heart rate than did younger subjects during mental arithmetic performance. Since change in heart

rate is an index of arousal, these data support the notion that for optimal learning elderly subjects need more arousal than do younger individuals.

Annual Report of the Clinical Physiology Branch, NIA
July 1, 1975 through June 30, 1976

SUMMARY

The Clinical Physiology Branch is responsible for the maintenance of the Baltimore Longitudinal Study of Aging for the Intramural Program and conducts research on the physiological processes of aging. Where feasible, man is considered the species of choice, but many correlative studies are conducted in the rat and dog as well.

A summary of the current status of the Longitudinal Study shows that 1,050 participants have had a total of 5,547 visits since 1958. Of these subjects, 555 were tested 5 or more times and 134 were tested 10 or more times. There have been 147 deaths and 250 have withdrawn from the study over its 18 year history. The active sample is thus at its goal, 653 men.

The unique nature of the participants in this study made it necessary to examine the characteristics of the participants at successive 2 year cycles in order to verify the impression that, despite an experimental design which requires continued recruitment, new subjects, introduced into the study remain similar over the time course of the study. Therefore subjects who were tested during each of the 8 two-year cycles were compared. Subjects remained remarkably similar with respect to mean age, self-reported health status, education, economic status, marital status, religious affiliation, social adjustment, and general symptomatology. Thus the generally high socioeconomic, health, and marital status of the population has been consistently maintained.

Further understanding of the known decline in basal metabolic rate (BMR) with age has been gained through a study which correlated the BMR with a measurement of muscle mass, the 24 hour urinary excretion of creatinine. Earlier studies from this laboratory showed that the fall in BMR was not attributable to a decline in thyroid function but was instead secondary to a loss of cellular mass with age. The creatinine excretion data now takes this biological fact a step further and demonstrates that essentially all of the fall in oxygen consumption with age is explainable on the basis of the specific loss of skeletal muscle mass.

Further analysis of the sexual characteristics of the Longitudinal Subjects has brought out several interesting facts which should assist in the evaluation of human sexuality factors. Surprisingly, the age at first coitus correlated strongly negatively with the number of sexual partners prior to age 40. It has been generally assumed that these characteristics would be positively related. Thus from an epidemiologic standpoint, the age of first coitus provides a measure of maximal incubation time for a potentially venerally transmitted agent, while the number of partners provides a measure of probability of infections. Both factors need to be considered independently.

A new study of osteoarthritis has been initiated. Advantage was taken of the fact that hand roentgenograms had been obtained longitudinally in

order to quantify the presence of osteoporosis and lateral chest x-rays have been obtained as part of the general medical examination. Thus osteoarthritic changes in the hands and in the thoracic vertebrae could be independently measured. The hand films have been meticulously analyzed and provide for the first time detailed information on the exact rate of progression of the osteoarthritic process and on the hierarchy of specific joints involved. Osteoarthritis is a serious disorder of aging which has in the past received very little research interest.

Further understanding of the susceptibility of older patients to life-threatening disturbances of water and salt balance has been gained by studies of the sensitivity of the hypothalamic-posterior pituitary axis to an increase in serum osmolality. By imposing a carefully controlled stress on the endocrine system responsible for the secretion of the antidiuretic hormone, arginine vasopressin, studies on the participants in the Longitudinal Study of Human Aging show a remarkable and unexpected increase with age in the sensitivity of the system. The stress used was an increase in serum osmolality created by the controlled infusion of hypertonic saline. The very high plasma levels of hormone resulting from this test in older subjects resulted however in nearly identical physiological responses by the kidney in conserving water. Thus the well-known loss of renal function with aging is compensated by an over-reaction of the hormonal water-saving mechanism. This compensatory endocrine response can itself cause serious clinical problems in the so-called "inappropriate vasopressin secretion" disturbances which commonly occur in older patients.

In vitro studies of basic biological aging processes have generally been undertaken on tissues from experimental animals. Species and even strain differences in the effects of aging are well known and therefore studies of these processes in man ultimately must be accomplished. Among the tissues which could be readily available for such studies in man are the leukocytes in peripheral blood and adipocytes in fat tissue removed at surgery. Studies on human leukocytes have just started and important progress has been made in the study of the human fat cell membrane. The enzyme adenylate cyclase (AC) is present in the cell membrane and is responsible for the production of cAMP from ATP. Numerous hormones initiate their action by activating AC; the cAMP formed in the "second messenger" which then produces the unique hormonal effects on the cell. The rat AC enzyme has been well-characterized, but the characteristics of the human enzyme and of the conditions under which its activity could be studied in vitro were poorly understood. Studies on fat cells from 50 subjects have now shown that response of the cell to epinephrine is not seen unless a guanine nucleotide analog (GMP-PNP) is added. The temperature and pH response characteristic of the enzyme have also been delineated. These fundamental studies lay the groundwork for characterization of the effects of aging, of sex differences, and of important clinical conditions on cell function in man.

In rat liver, AC activation by epinephrine is greatly increased in old (24 mo) as compared to mature (12 mo) animals. Since this is a cell membrane-bound enzyme, the stability of the enzyme in homogenates was studied, and this was found to be significantly decreased in the old animals.

These results are in agreement with other studies on a different group of cell membrane bound enzymes, the ATPases. In this case, the age changes were shown to be related to altered phospholipid (PL) content of the membranes. Since activity of AC is also dependent on membrane PL, this suggests that alterations in lipid metabolism of liver membranes may be important in aging. Evidence has also been obtained that these age changes can be influenced by nutritional factors.

Another major effort continues in the study of the specific hormonal receptor molecules on the cell surface and in the interior of the cell. This first step in hormonal action has now been extended from previously studied tissues (adipose tissue, liver, splenic lymphocytes, neurones) to myocardial cells. The response of this specialized muscle to the catecholamines decreases with age. Receptors for these hormones have now been characterized for the rat myocardium since the effect of aging on biophysical properties of this tissue are currently under study. These rat receptors closely resemble those previously described in the dog. Aging results in a significant reduction in the concentration of receptors but the affinity of the receptor for its hormone is unchanged by age. Thus the decreased physiological effect of the hormone is due to a quantitative loss in receptors, but not in a qualitative change.

There have been further advances in the delineation of the role of the recently described hormone, gastric inhibitory polypeptide (GIP). Previous studies from our laboratory demonstrated that the time course of the appearance of this hormone in the blood stream in response to glucose ingestion closely matches the time course of the increase in insulin levels when the blood glucose concentration is "clamped" at a constant hyperglycemic level. We have now examined the effects of three conditions associated with poor glucose tolerance: diabetes mellitus, aging, and obesity. The GIP response in these three states differ. Aging has no effect on the GIP response. Diabetics have abnormally high fasting levels but their response to oral glucose is reduced. GIP levels in obesity are elevated but the response to oral glucose is appropriate. The glucose clamp technique also permits quantification of the sensitivity of the pancreatic beta cell to the endogenously released GIP. The aging beta cell shows a distinct loss of sensitivity to GIP which is not seen in diabetes or in obesity.

We have also been able to identify and study an interesting group of 8 subjects with greatly disparate performance on their intravenous and oral glucose tolerance tests, with much higher tolerance (i.e., better performance) on the oral test. The mechanism of this disparity is now better understood since the glucose clamp studies demonstrate that they have elevated GIP levels basally and have a further augmented GIP response to oral glucose. Thus GIP physiology may play a role in normalizing the metabolism of glucose by augmenting the insulin-releasing effects of hyperglycemia in subjects who would otherwise have an inadequate insulin output.

Studies of cardiovascular changes with age have continued along two major lines of investigation. The first is toward an understanding of the molecular mechanism of age-associated changes in myocardial contractility, contractile function, and inotropic responsiveness of isolated rat cardiac

muscle. The second is toward an understanding of the changes in function of the intact cardiovascular system in both animal models and in man. Thus the rat isolated muscle preparations provide predictions for the intact heart which can be tested in man or in the dog when more invasive methods need to be used.

The studies of the dynamic stiffness properties of left ventricular trabeculae carneae from aged rats confirm the presence of increased diastolic or resting stiffness at L_{max} in muscles from old rats. These studies more importantly demonstrate an age-associated increase in stiffness of trabeculae carneae during the isometric twitch. The increase in left ventricular stiffness during contraction may account for the disparity noted by many groups of investigators between the age-associated decrease in V_{max} (velocity of fiber shortening at zero load) and the lack of age change in the maximal rate of isometric force development. What is being postulated here is that there is a decrease in active state intensity with age reflected in the decrease in V_{max}. Maximal rate of tension development, dT/dt, under isometric conditions reflects not only the intensity of active state but also the elastic properties of the entire piece of muscle under study. If there is an increase in stiffness in the muscle from older rats this would compensate for the decrease in active state and result in no change in peak isometric dT/dt with age.

Initial studies in the intact dog heart under anesthesia suggest a marked age-associated decrease in chrono-tropic response of these dogs to isoproterenol and the catechol releasing agent tyramine. These observations raise the possibility that the explanation for the age-associated decrease in maximal heart rate response to exercise may be related to both decreased intrinsic catecholamines available for release and a decreased responsiveness of the sinus node to catecholamine stimulation. These observations will be pursued in the unanesthestized animal in the near future.

Echocardiographic studies have now been completed in 105 participants in the Baltimore Longitudinal Study of Aging. These studies demonstrate that this non-invasive technique is sensitive enough to identify age differences in the left ventricular wall (thickness increases with age), in the aorta (diameter increases with age), and in mitral value motion (the rate of mid-diastolic value closure decreases with age). Since the size of the ventricular cavity does not differ with age, there is probably an increase in left ventricular wall mass with aging, as there is in the rat heart. The value motion differences suggest a decrease in left ventricular filling rate with age probably as a result of delayed relaxation or of alteration in left ventricular diastolic compliance. The significant age differences obtained on the heart under resting conditions have led to the design of more challenging studies of cardiovascular function in man under conditions of physical and pharmacological "stress". Fundamental mechanisms underlying the significant age differences may thus be delineated in man.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00001-06 CPB

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)
Metabolic studies of aging in man

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. Andres	Chief, Clinical Physiology Br.	CPB	NIA
	J. D. Tobin	Medical Officer	CPB	NIA
	E. A. McGuire	Staff Fellow	CPB	NIA
	G. S. Raizes	Clinical Associate	CPB	NIA
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	D. Elahi	Staff Fellow	CPB	NIA
OTHER:	M. Berman	Chief, Lab. of Theoretical Biology	LTB	NCI

COOPERATING UNITS (if any)
Laboratory of Theoretical Biology, National Cancer Institute, Bethesda Maryland

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Metabolism Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.5	2.5	2.0

SUMMARY OF WORK (200 words or less - underline keywords)

This project is primarily concerned with furthering our understanding of the relations between physiological aging processes and specific diseases in the elderly. The major focus is on glucose homeostasis and diabetes mellitus. Studies are directed at long-term follow-up of volunteers in the Baltimore Longitudinal Study of Aging in order to acquire actuarial data for judging the significance of varying levels of performance on the diagnostic tests for diabetes mellitus which are in clinical use. Other studies are directed at discovering the patho-physiologic mechanism underlying the age changes in performance on these tests.

PHS-6040
(1-75)

Project Description:

Objectives: This project is primarily concerned with furthering our understanding of the relations between physiological aging processes and specific diseases in the elderly. The major focus is on glucose homeostasis and diabetes mellitus. Studies are directed at long-term follow-up of volunteers in the Baltimore Longitudinal Study of Aging in order to acquire actuarial data for judging the significance of varying levels of performance on the diagnostic tests for diabetes mellitus which are in clinical use. Other studies are directed at discovering the patho-physiologic mechanisms underlying the age changes in performance on these tests.

Methods Employed: Standard tests (oral and intravenous glucose tolerance, cortisone-primed oral glucose tolerance, and intravenous tolbutamide response tests) are given on a rotating basis on consecutive visits. The glucose-clamp technique has been developed for controlling the blood glucose concentration with servo-control principles. Immunoreactive assays for insulin and glucagon are used. Tracer-labeled glucose is used to measure endogenous glucose production. Kinetic modeling is done on a UNIVAC 1108 computer using the SAAM program of Berman and Weiss.

Major Findings: In preparation for a major analysis of longitudinal data on glucose metabolism, serum lipids, body composition (obesity), and dietary and activity variables, a major review of our clinical classification of participants on each visit from the inception of the study in 1958 through June 30, 1975, was undertaken. The emphasis has been on the classification for coronary heart disease (by history and electrocardiographic data), for renal disease, and for history of medications which could influence any of the physiological variables of interest (glucose tolerance, serum lipids, blood pressure, for example). This review will be completed by June 30, 1976.

A variety of manuscripts were prepared and published (see below) and six other manuscripts in the metabolic area have been submitted for publication. This diverse project then has primarily been concerned this year with manuscript preparation and with the clinical classification chore.

In addition three new projects are under way. (1) With the collaboration of Dr. George Roth of the Endocrine Section a study of the effect of aging on hormonal receptors on human circulating mononuclear cells is underway. The initial hormones being investigated are beta adrenergic agents and insulin. (2) The amino acid arginine occupies a potentially strategic location in the insulin secretogagues, glucagon and gastric inhibitory polypeptide, and is itself a beta cell stimulator. A study of the effect of aging on arginine effects on insulin secretion has been started as well as on the interactions of these several secretagogues in the maintenance of glucose and insulin homeostasis. (3) The glucose clamp technique is being used in a study of the mechanism underlying the thiazide effect on the production of glucose intolerance (primary drug effect vs. secondary effect of drug-induced hypokalemia).

Significance to Bio-Medical Research and the Program of the Institute. The remarkable prevalence (50%) of abnormal glucose tolerance in the older population of the United States coupled with the increased morbidity and mortality of patients with true diabetes mellitus demands a delineation of the effects of aging on the pathophysiology of carbohydrate homeostasis.

Proposed Course of the Project: The large-scale longitudinal analysis of carbohydrate data and correlations with metabolically and clinically related variables will be accomplished.

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Rowe, J. W., Shock, N. W., DeFronzo, R. A.: The influence of age on the renal response to water deprivation in man. Nephron (in press).

McGuire, E. A. H., Helderman, J. H., Tobsin, J. D., Andres, R., and Berman, M.: Effects of arterial versus venous sampling on the analysis of glucose kinetics in man. J. Appl. Physiol. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00002-14 CPB

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)
The effect of age on the gastrointestinal mediation of insulin release

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. K. Andersen	Clinical Associate	CPB	NIA
	J. D. Tobin	Medical Officer	CPB	NIA
	R. Andres	Chief, Clinical Physiology Br.	CPB	NIA
OTHER:	J. C. Brown	Univ. of British Columbia, Vancouver, B.C., Canada		

COOPERATING UNITS (if any)
Department of Physiology, University of British Columbia, Vancouver, British Columbia, Canada

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Metabolism Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.7	3.0	2.7

SUMMARY OF WORK (200 words or less - underline keywords)

This study was designed to examine generally the influence of the gastrointestinal tract upon the insulin response to ingested glucose, and specifically the role of one or more gastrointestinal hormones in the process of insulin secretion. The study is being applied to altered physiologic states, such as aging and obesity, as well as pathologic states of diabetes, and reactive hypoglycemia. It is well established that the response of the endocrine pancreas to ingested nutrients such as glucose is greater than its response to the same stimulus administered intravenously. The physiologic and biochemical etiology of this finding, however, has remained open to debate and investigation. Similarly, the significance of this process in pathologic states is unknown. While gastrointestinal hormones have been suggested as possible mediators of the phenomenon, until recently none has been clearly shown to play a critical role. The development of a sophisticated laboratory technique for studying this process, and the availability of a specific assay for a newly discovered gastrointestinal peptide which appears a likely candidate as the sought-after mediator, have provided the essential means of investigating this process. The present studies show that a specific hormone, gastric inhibitory polypeptide (GIP), responds to oral glucose with a time course in plasma insulin concentration. Furthermore there are significant differences in the physiology of GIP with aging, obesity, and diabetes.

Project Description:

Objectives: This study was designed to examine generally the influence of the gastrointestinal tract upon the insulin response to ingested glucose, and specifically the role of one or more gastrointestinal hormones in the process of insulin secretion. The study is being applied to altered physiologic states, such as aging and obesity, as well as pathologic states of diabetes, and reactive hypoglycemia. It is well established that the response of the endocrine pancreas to ingested nutrients such as glucose is greater than its response to the same stimulus administered intravenously. The physiologic and biochemical etiology of this finding, however, has remained open to debate and investigation. Similarly, the significance of this process in pathologic states is unknown. While gastrointestinal hormones have been suggested as possible mediators of the phenomenon, until recently none has been clearly shown to play a critical role. The development of a sophisticated laboratory technique for studying this process, and the availability of a specific assay for a newly discovered gastrointestinal peptide which appears a likely candidate as the sought-after mediator, have provided the essential means of investigating this process.

Methods Employed. The hyperglycemic and euglycemic clamp techniques have been used to maintain human subjects at preselected elevated or normal blood glucose levels. This allows evaluation of factors affecting insulin secretion in the absence of changing blood glucose concentration. In addition, by maintaining stable glucose concentrations before and after ingestion of insulinotropic agents, the effect of the gastrointestinal influences may be specifically quantified. This allows precise determination of the sensitivity of endocrine cells in the gastrointestinal tract, the sensitivity of the pancreatic beta-cells to the gastrointestinal hormone(s), and the factors which affect the response of these tissues.

The hyperglycemic clamp study is performed by administering a calculated intravenous glucose load sufficient to raise the blood glucose level to a specific desired level. By using an automated, rapid blood glucose analysis technique, changes in the intravenous infusion are made every four minutes to insure a stable blood glucose level. After one hour of intravenous glucose infusion alone, a standard dose of an oral glucose or other solution is ingested by the subject, and the subsequent blood glucose level is maintained at the previous level.

In the euglycemic clamp study, the subject's normal, fasting blood glucose level is maintained by means of a combined insulin and glucose infusion, with the glucose infusion varying as before depending on the blood glucose concentration. The insulin infusion is given as a primed-continuous infusion, designed to provide a rapid square-wave elevation of insulin to a preselected level. After one hour of maintaining stable glucose blood levels, the standard oral dose is given, and the euglycemic levels are maintained.

The subjects for study are primarily members of the Longitudinal Study, supplemented by some volunteer participants. The subjects are classified

according to previous glucose tolerance (both oral and intravenous), age, obesity, medical and family history. Both insulin and gastric inhibitory polypeptide (GIP) are determined by radioimmunoassay of plasma samples. Lyophilized plasma samples are saved for further hormone assays.

Major Findings: When oral glucose is administered while the blood glucose is maintained at basal euglycemic levels, no insulin release is seen, despite the typical GIP response to oral glucose. This suggests that GIP acts to augment insulin secretion only in the presence of hyperglycemia. Further studies to identify this process reveal that the threshold for GIP's insulinotropic action is at a blood glucose level 20-30 mg% above fasting levels.

While aging has been shown to result in decreased insulin responsiveness to glucose (administered both orally and intravenously), no data have previously been presented regarding the possible role of the of the gastrointestinal medication of this process. Our studies show that the response of GIP to oral glucose is unchanged with aging, but there is a signficiant decrease in the pancreatic sensitivity to endogenous GIP, resulting in diminished insulin release.

Obesity has also been known to affect the insulin response to glucose administration, with higher insulin levels seen after both oral and intravenous glucose. GIP appears to be similarly associated with obesity, in that higher GIP levels are seen initially, and the response to oral glucose is greater than in lean, age-matched individuals. Pancreatic beta-cell sensitivity to endogenous GIP is normal in obesity, thus confirming that the physiological alteration in obesity is primarily at the peripheral insulin interaction site.

In diabetes, endogenous GIP is present in high basal amounts, but is increased by oral glucose to a far lesser degree than in non-diabetic subjects. Pancreatic sensitivity to the change in GIP is comparable to normal subjects, however, with the final result being the marked decrease in insulin produced in the diabetic subjects following oral glucose.

There are individuals who exhibit considerable disparity in their glucose tolerance measured by intravenous versus oral glucose loading. In individuals who have significantly better glucose tolerance after oral administration, compared to I.V. glucose loading, the response of endogenous GIP to oral glucose appears greater than normals (persons with comparable intravenous and oral glucose tolerance) with the result that the normal insulin response to oral glucose is augmented.

Other nutrients, such as fat, are also capable of stimulating GIP secretion and therefore insulin release. A comparison of matched doses of oral glucose and an oral fat mixture reveals that GIP is secreted more slowly following fat ingestion, and results in similar insulin augmentation as that seen after oral glucose. Studies to examine the relationship of glucose- and fat-induced GIP release, and their effectiveness on beta-cell function, are

currently underway.

Significance to Bio-Medical Research and the Program of the Institute: The high prevalence of altered glucose tolerance in aging and obesity, as well as the high incidence of adult-onset diabetes mellitus require further understanding of factors which contribute to this process. In addition, an understanding of similarities and differences in the pathophysiology of the various categories of glucose intolerance provides the hope for more and improved methods of treatment. Pathological states associated with alterations in gastrointestinal hormones are only superficially understood currently, and further investigation of these hormone systems adds greatly to a young area of medical knowledge.

Proposed Course of Project: Alterations in normal physiology (aging, obesity), metabolism (diabetes, reactive hypoglycemia, glucose tolerance disparity), and other pathological conditions (surgical intervention in gastrointestinal continuity of the small bowel, diseases affecting the structural continuity of the small bowel) are being studied. Mechanisms of GIP-beta cell interaction, alterations in GIP release by various stimuli, and relationship to antecedant diet and drug therapy, are currently underway. The role of other hormones, such as glucagon, is also being studied.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00004-03 CPB

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)
Ethanol metabolism: The effect of age on the pharmacokinetics of ethanol in man

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. D. Tobin	Medical Officer	CPB	NIA
	R. Andres	Chief, Clinical Physiology Br.	CPB	NIA
OTHER:	E. A. McGuire	Staff Fellow	CPB	NIA
	R. E. Vestal	Research Fellow, Vanderbilt U. School of Medicine		
	E. Mezey	Chief, Hepatology, Baltimore City Hospitals		
	M. Berman	Chief, Lab. of Theoretical Biology	LTB	NCI

COOPERATING UNITS (if any)
Division of Clinical Pharmacology, Depts. of Medicine & Pharmacology, Vanderbilt University, School of Medicine, Nashville, TN 37203
Alcohol Research Unit, Division of Gastrointestinal, Baltimore City Hospitals
National Cancer Institute, Bethesda, Maryland

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Metabolism Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this study was to examine under conditions of acute ethanol administration the effect of age on: (1) the kinetics of distribution and elimination of ethanol, (2) posterior pituitary function and free water clearance, (3) psychomotor and cognitive performance. Ethanol concentrations in the blood reach slightly higher levels in older subjects even though standardized amounts are administered to all subjects. This is attributable to the change in body composition (loss of cellular mass) with aging. The rate of ethanol metabolism does not change with age.

PHS-6040

Project Description:

Objectives: The purpose of this study was to examine under conditions of acute ethanol administration the effect of age on: (1) the kinetics of distribution and elimination of ethanol, (2) posterior pituitary function and free water clearance, (3) psychomotor and cognitive performance.

Methods Employed: Ethanol was administered intravenously as a 15% solution in a dose of 400 mg/m^2 surface area/min over 60 minutes following an overnight fast. Blood ethanol concentrations were measured by gas liquid chromatography at fixed intervals during and for 4 hours after the infusion. The SAAM-26 computer program is being used to develop a compartmental model to describe the kinetics of distribution and elimination.

Major Findings: The experimental studies were completed last year. Analyses this year showed that although the rate of elimination of ethanol is unchanged with age, the blood levels of ethanol achieved were significantly higher in the older subjects. It is of importance that, in an effort to compensate for the varying degrees of obesity in a healthy population, ethanol dosage was computed using body surface area as a reference base. While this is a reasonable decision, the ethanol "space", essentially total body water, still is smaller per unit of surface area in older subjects than in younger. Thus the loss of cell mass with aging will in itself cause somewhat higher drug levels when apparently identical doses of the drug are administered.

Significance to Bio-Medical Research and the Program of the Institute. The changes in body composition with aging can influence pharmacokinetic estimates of drug metabolism. These studies point to the fact that clinical pharmacologic studies in aging need to be interpreted with full knowledge of the physiological alterations that accompany aging.

Proposed Course of Project: The studies are completed, and the final manuscript has been prepared.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00005-03 CPB

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)
Hypothalamic-hypophyseal responsiveness and aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. D. Tobin	Medical Officer	CPB	NIA
	R. Andres	Chief, Clinical Physiology Br.	CPB	NIA
OTHER:	J. H. Helderman	Research Fellow, Peter Bent Brigham Hospital		
	R. E. Vestal	Research Fellow, Vanderbilt U. School of Med.		
	Gary L. Robertson	Chief, Endocrinology Section, VAH, Indianpolis		

COOPERATING UNITS (if any)
Peter Bent Brigham Hospital, 721 Huntington Ave., Boston, MA 02115
Division of Clinical Pharmacology, Depts. of Med. & Pharmacology, Vanderbilt University School of Medicine, Nashville, TN 37203
VA Hospital, Indiana U. School of Medicine, Indianapolis, IN

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Metabolism Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.3	0.1

SUMMARY OF WORK (200 words or less - underline keywords)
These studies are designed to ascertain the effect of aging on hypothalamic-hypophyseal secretion of the antidiuretic hormone, arginine vasopressin (AVP), in response to: 1) overnight dehydration, 2) hypertonic saline infusion and 3) intravenous ethanol infusion. These standardized tests of both negative (ethanol) and positive (hypertonic saline) stimuli to the hypothalamic-hypophyseal axis showed: (1) with aging there is an enhanced sensitivity of AVP secretion to hyperosmolality; (2) the inhibitory effects of ethanol on AVP do not change with age during the first 30 minutes of the infusion, then there is a paradoxical loss of the inhibition in older subjects; (3) the transient effect of ethanol in the aged is probably due to the increase in serum osmolality induced by the ethanol, thus secondarily causing a counter-effect of AVP secretion. The increased sensitivity of this endocrine mechanism with age is probably an important factor in the known susceptibility of older patients to the development of serious hyperosmolality syndromes.

PHS-6040
(1-76)

Project Description:

Objectives: These studies are designed to ascertain the effect of aging on hypothalamic-hypophyseal secretion of the antidiuretic hormone, arginine vasopressin (AVP), in response to: 1) overnight dehydration, 2) hypertonic saline infusion and 3) intravenous ethanol infusion. From such studies mechanisms of aging changes will be described and some of the clinical problems in salt and water conservation in aging individuals may be explained.

Methods Employed: The hormone arginine vasopressin was measured by radioimmunoassay of acetone-extracted plasma utilizing the Glick-1 (G1-1) antiserum. The technique is highly specific as it can distinguish AVP from all other naturally synthesized and secreted posterior pituitary peptides. Samples of plasma obtained during the various perturbations were rapidly frozen and assayed at a later date.

In all studies subjects across the age spectrum were carefully selected to eliminate those who had cardiac or renal diseases and those taking alcohol or drugs. They refrained from fluids overnight. Plasma samples obtained just prior to a perturbation thus represented the AVP response to mild overnight dehydration. In one group of studies 3% NaCl was infused by vein over 2 hours following a 20 minute basal period in the recumbent position. Plasma samples were obtained during the infusion for plasma AVP, sodium, and osmolality. Urines were collected before and after the infusion for the measurement of sodium and osmolality. The goal of these studies is to provide a hyperosmolar stimulus which is recognized by the hypothalmus and leads to posterior pituitary release of antidiuretic hormone.

In another group of studies 15% (v/v) ethyl alcohol was infused intravenously at the rate of 400 $mg/m^2/min$ over one hour. Blood samples for AVP, ethanol, sodium, and osmolality were obtained at intervals over 5 hours. As ethanol has been implicated in diminishing or abolishing secretion for AVP, it provided a negative stimulus to test the hormonal axis.

Urine and plasma measurements of osmolality and volume allowed computation of osmolar clearance and of free water clearance which quantify the physiologic response to the AVP present in the blood.

Major Findings: The ethanol infusion studies were completed last year, and the hypertonic saline infusion studies were completed this year. The saline infusion resulted in linear increases in serum osmolality which were nearly identical in older and younger subjects; thus the stimulus to AVP secretion did not differ with age. The sensitivity of the hypothalamic osmoreceptor to hyperosmolality can be computed from the slope of the serum AVP concentration on the serum osmolality. Surprisingly there was clear-cut evidence of increased sensitivity of the receptor in the older subjects; AVP levels rose much higher.

The physiologic effect of the released antidiuretic hormone was assessed by computing the change in free water clearance by the kidney. Despite higher AVP levels in the older subjects, the changes in free water clearance were

identical in the two age groups. Thus a diminished renal capacity to respond to AVP is compensated for by a greater release of the hormone in older subjects.

These studies help to explain the apparently paradoxical time course of serum AVP response on the intravenous ethanol test. We previously showed that in younger subjects there was a progressive fall in AVP as serum ethanol levels rose; in older subjects the fall in AVP was transient and levels began to increase although serum ethanol continued to rise. The results are understandable on the basis of a secondary ethanol effect on plasma osmolality; as osmolality increases the inhibitory effect of ethanol on the hypothalamus conflicts with the stimulatory effect of the rising osmolality. In older subjects, the increased sensitivity of the hypothalamus to hyperosmolality reverses the ethanol inhibition.

Significance to Bio-Medical Research and the Program of the Institute: There are clinical indications that the aged individual has a diminished ability to maintain salt and water homeostasis. Studies performed here in the past have examined the end organ phenomena that pertain to salt and water balance. Analysis of the central mechanisms involved is required to understand fully the impact of aging on the ability to maintain the internal milieu. Such understanding may alter clinical decisions about fluids and medications prescribed for the elderly patient.

Proposed Course of the Project: The project has now been completed, and a final manuscript has been completed.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00006-03 CPB

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)
Antipyrine metabolism in man: Influence of age, alcohol, caffeine, and smoking

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	*R. E. Vestal	Research Fellow, Vanderbilt U. School of Medicine		
OTHER:	A. H. Norris	Chief, Human Performance Sec.	CPB	NIA
	J. D. Tobin	Medical Officer	CPB	NIA
	**B. H. Cohen	Professor of Epidemiology, JHU		
	N. W. Shock	Chief, Gerontology Res. Ctr.		NIA
	R. Andres	Chief, Clinical Physiology Br.	CPB	NIA

COOPERATING UNITS (if any)
*Division of Clinical Pharmacology, Depts. of Medicine & Pharmacology, Vanderbilt University School of Medicine, Nashville, TN 37203
**Department of Epidemiology, School of Hygiene & Public Health, Johns Hopkins University

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Metabolism Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

SUMMARY OF WORK (200 words or less - underline keywords)

Antipyrine is a "marker" for a family of drugs metabolized by hepatic enzymes. Aging subjects show a slower degradation rate of the drug than younger subjects. This age effect is however mainly secondary to the fact that older subjects smoke fewer cigarettes and drink less caffeine (coffee and tea) than do younger subjects. Both of these habits could be shown by multivariate analysis to account for more of the variance in drug metabolism than aging per se. Thus in the evaluation of aging effects in man, differences could be erroneously attributed to "biological aging processes" which are in fact due to differences in habits or in behavior patterns of different age groups.

Project Description: This project has been completed.

Publications:

Vestal, R. E., Norris, A. H., Tobin, J. D., Cohen, B. H., Shock, N. W., and Andres R.: Antipyrine metabolism in man: Influence of age, alcohol, caffeine, and smoking. Clin. Pharm. Therap. 18: 425-432, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00008-04 CPB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Age Effect on Intrinsic Cardiac Muscle Regulation and Neural Control of Heart and Circulation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. A. Spurgeon	Staff Fellow	CPB	NIA
	F. C. P. Yin	Clinical Associate	CPB	NIA
Other:	J. Froehlich	Medical Officer	LMA	NIA
	M. L. Weisfeldt	Chief, Div. of Cardiology, JHU		
	H. L. Greene	Asst. Prof. of Medicine, JHU		
	N. W. Shock	Chief, Gerontology Research Center		NIA

COOPERATING UNITS (if any)

Division of Cardiology, Department of Medicine, Johns Hopkins University

LAB/BRANCH

Gerontology Research Center, Clinical Physiology Branch

SECTION

Cardiovascular Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.85	.85	1.0

SUMMARY OF WORK (200 words or less - underline keywords)

This project is a broad multifaceted program which attempts to characterize and quantify the changes in cellular and organ physiology and pharmacology which occur with advancing age. A major goal of the program is to project the findings of one discipline in terms of the meaning to the whole system by measuring a spectrum of indices of cardiac performance at several levels of integration on the same tissue samples. Thus one is better able to assign some relative value to each measured reaction or cellular event in terms of the overall control of cardiac function. Neural, pharmacological, and physiological techniques are employed extensively from a functional level, while biochemical assay techniques are used to identify cellular change.

PHS-6040
(1-75)

Project Description:

Objectives: The broad goals of this series of projects is to identify and characterize the age-induced effects on the physiology and pharmacology of cardiac muscle, the circulatory system, and the neural regulation of the cardiovascular system. The section has previously documented some of the organ changes associated with aging, and these continue as the basis for current and future protocols. The project strives wherever possible to correlate the subcellular, cellular, organ, and organismic responses to provide an integrated picture of cardiovascular regulation in terms of its meaning to overall heart performance.

Methods Employed: Detailed methods for biochemical investigations and for isolated rat muscle preparations have appeared in previous reports. Investigations on dogs are carried out using standardized pharmacologic protocols.

Studies are underway relating age-associated calcium uptake and mechanical performance with both assays performed in the same heart. Hypertrophy as a possible determinant of age-associated cardiac changes is being investigated at the model development level with a planned expansion into the cellular mechanistic and mechanical performance levels. An extensive series of experiments in cardiac regulation and control have been initiated in dogs, where the relative size and amount of heart tissue available for isolated tissue and cellular investigations is more conducive to interdisciplinary studies. Reflex control of heart rate, pharmacologic interaction of neurotransmitter ionotropes, and reflex control of pressure have been investigated across the entire adult life span of the dog, and biochemical as well as isolated tissue experiments on this same population of animals has begun. This same group of animals have served as the subjects of an electrophysiologic study regarding the special conduction tissues and pacemaker activity of the aged heart. Conduction velocities, refractory periods, and maximum excitable rates have been examined.

Major Findings: The major findings of this series are concerned with the role of calcium in the aging response of the rat heart. There is a clear age difference in the calcium uptake velocity which correlates with timing of the mechanical events of isolated muscle tension development. Age decreases the activity of the calcium system and prolongs the contraction time of the isolated muscle. Calcium binding, which is thought to govern the relaxation phase of contraction, would be expected to decrease in parallel with increases in contraction time if the two were related. Although the correlative projects with the dog as an aging model are well underway, with the initial phases of the study completed, the analysis of results depends largely on the completion of all phases of the protocol before final correlations can be made. However, the aged heart shows a definite decrease in catecholamine sensitivity relative to young controls. This would indicate that the aged individual's nervous system would be relatively less effective for a given discharge level as compared to that of a young control. Decreased neural control would then be a natural consequence.

Significance to Biomedical Research and to the Program of the Institute: It appears that the major changes associated with cardiac aging are those of regulation and control. This is true at the cellular level in the case of SR calcium handling ability, and appears also to be the case at the organ level in, for example, the regulation of heart rate by the sympathetic nervous transmitter. In addition, a decreased regulatory ability of the total organism is implied by the decrease in heart rate responsiveness to artificial reduction in baroreceptor pressure seen in the aged population. Other reductions, such as the reduced ability to respond to digitalis preparations listed in last year's report support this concept. The direction of this section in general and this project in particular has been to carry out an integrated approach to the problems of aging within the context of an organ system approach. Therefore, the real significance of this project to date has been the synthesis of information at the subcellular, cellular, and organ levels as it interrelates with aging in general.

Proposed Course of the Project: This project has to date identified several major areas which are potentially useful for further investigation. Cardiac receptor pharmacology and physiology seem especially affected by the aging process. Further clarification of the nature and extent of changes in this major component of cardiovascular control seem warranted. Further investigation into the nature of calcium release responsible for the initiation of contraction would be a necessary next step following the characterization of the uptake mechanism. Reflex control in general must also be followed in an aging context, a subject which the present study only touches on. The whole question of the nature, degree, and effect of hypertrophy of the heart with age needs clarification. Induction of hypertrophy in the young adult has promise here.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00009-02 CPB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Dynamic Mechanical Properties of Aged Myocardium

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. A. Spurgeon	Staff Fellow	CPB, NIA
	F. C. P. Yin	Clinical Associate	CPB, NIA
	P. T. Thorne	Head, Technical Development	OC, NIA
OTHER:	W. Milnor	Prof. of Physiology	JHU
	M. L. Weisfeldt	Chief, Div. Cardiology	JHU
	N. W. Shock	Chief, Gerontology Research Center	NIA

COOPERATING UNITS (if any)
Department of Physiology and Division of Cardiology, Department of Medicine, Johns Hopkins University

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Cardiovascular Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

SUMMARY OF WORK (200 words or less - underline keywords)

Mechanical properties of heart muscle determine to a great extent the way in which biochemical events in the cell are transduced into muscle tension and ultimately into development of pressure in the heart. Changes in the mechanical properties of the aged heart could account for the observed changes in cardiac performance as determined by a variety of indices. This program uses vibration techniques to measure the components of a cardiac and vascular muscle which determine the overall "stiffness", and measures the age induced effects on stiffness produced by regulatory events of both neurohumoral and mechanical origins. As the major elements of cardiac control, these events, and their interaction with the mechanical properties of the heart form the basis for cardiac control.

PHS-6040
(1-75)

Project Description:

Objectives: The goals of this study are to identify, correlate and quantify the relative roles of the mechanical and metabolically -generated compartments of force generation in cardiac muscle which are associated with a decline in active state. Data from this section indicates that the prolonged active contraction seen in aged myocardium may be due to an increase in the time required to dissipate the active state or in a delay attributable to an increase in the mechanical stiffness of the muscle. Changes in stiffness could be expected to alter the speed and the absolute amount of force delivered to the ends of the muscle fibre. The aged heart depends on the hydraulic impedance to blood flow exhibited by the aorta, and stiffness of the major vessels of the heart is at least as important in determining the overall cardiac performance of the aged heart as the properties of the muscle itself. The impedance (dynamic pressure flow relationship) of the aging rat aorta is being investigated in the context of a model of the aging process of the major arterial vessels. The overall objective of this entire series of studies remains to quantify and assess the effects of age on the mechanical properties of the myocardium and to define the relative roles of the major factors responsible for these changes.

Methods: Isolated superfused trabeculae carneae from adult and senescent rats are clamped in an apparatus which constrains the muscles to an isometric length. Using techniques developed for vibration analysis, the stiffness of the sample of cardiac muscle is determined as a stress/strain ratio by subjecting the isometric muscle to sinusoidal length perturbations, typically of 0.022 mm, at frequencies from .001 to 100 Hz. The muscle is caused to contract isometrically during the study, enabling computation of the changes in dynamic stiffness associated with the contractile process. The resulting tension, which varies in step with the sinusoidal length changes imposed, is the strain produced stress, and by definition represents the total stiffness of the muscle.

The measurement of impedance is accomplished in much the same way, except the perturbation used in the contraction produced pressure of the rat heart. The pressure in the ascending aorta is measured by a catheter introduced via the carotid artery, while blood flow is measured by electromagnetic flowmeter. Using Fourier analysis to break the two signals into harmonic components and comparing the ratio of pressure to flow of each harmonic yields impedance information. Both this and the stiffness technique use perturbations and measure the result of those perturbations. Because hypertrophy and age may be the same stress, efforts to develop an "age hypertrophied" young heart are under way.

Major Findings: The initial study is now completed and prepared for publication. The study showed resting stiffness increased by about 25% with a similar increase in active contractile stiffness in senile rat myocardium as compared to young adult samples. It was further shown that the aged hearts are moving up a stiffer function curve which appears to be valid for both passive and active stiffness. Thus, the increased resting tension noted in past studies from this section appears to be the result of an increase in the intrinsic stiffness of the resting muscle, while the development of active contractile tension which appears undiminished in the old muscles is actually accomplished with a stiffer muscle. This suggests that the old heart muscle develops the requisite amount of tension with a reduced amount of muscle fibre shortening as compared to young rat myocardium.

The study of impedance of the aortic root has progressed to the pilot stage with the feasibility of measurement accomplished.

Muscle stiffness is also being measured in myocardium taken from senile dog hearts which have first been extensively studied for responsiveness to ionophores. This will provide data across species, and also provides coorelative data between structure and function on intact, cellular, and mechanical levels.

Significance to Biomedical Research and the Program of the Institute: The implications of the completed study have rather wide impact. First, the indication of increased stiffness in both passive and active states of the aged heart muscle suggests the age related change of this important measure occurs not only in the passive supportive structure of the muscle, but implies changes in the active contractile elements as well. Second, the suggestion that the old heart stiffness vs tension curve exhibits a steeper slope, and that this slope increases with age in both the passive and the active state indicates a fundamental change in the muscle with age. Third, although it is widely held that the old heart is incapable of extended performance under stress, the efficiency of the aged heart may actually be increased. Tension developed is the product of distance the contractile element shortens and the stiffness of the element. For tension to remain relatively unchanged across age, the amount of shortening required may decrease by as much as 25%. A similar shift in the aortic impedance would further modify the efficiency of cardiac performance. Although tension development fails to decrease markedly with age, the tension developing mechanism might well decrease, and the magnitude of that decrease may prove greater than suspected because of the "masking" effect of increased stiffness.

Since the aged heart appears to be hypertrophied compared to the young heart, one possible explanation for the differences in the mechanical properties observed is hypertrophy. Currently,

hypertrophy is thought to be produced by stress. Perhaps aging should be considered as a cardiac stress. A change in impedance of the aorta with age would certainly impose a physiological stress. Data regarding this influence are critical to understanding the aging process of the heart itself.

Proposed Course: 1). Investigate the relative roles of positive and negative ionophores on cardiac muscle stiffness. 2). Study paired potentiation and electromechanical coupling with special emphasis on the effects of delayed relaxation on mechanical potentiation. 3). Continue cross species verification of the universality of these observations as is currently under way in dogs. 4). Study the isolated aortic tissue in an analogous manner to the trabecular preparation after the impedance studies have been done on the intact animal with hopes of identification of the specific elements responsible for impedance change.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00010-03 CPB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Hemodynamics of the Left Ventricle and Mitral Valve in Aging Man

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	F. C. P. Yin	Clinical Associate	CPB, NIA
	G. Raizes	Clinical Associate	CPB, NIA
OTHER:	H. A. Spurgeon	Staff Fellow	CPB, NIA
	N. J. Fortuin	Asst. Prof. of Medicine	JHU
	M. L. Weisfeldt	Chief, Div. Cardiology	JHU
	N. W. Shock	Chief, Gerontology Research Center	NIA

COOPERATING UNITS (if any)
Division of Cardiology, Department of Medicine, Johns Hopkins University

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Cardiovascular Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.5	.3	.2

SUMMARY OF WORK (200 words or less - underline keywords)

Management, evaluation, and control of the aged heart require knowledge of the changes in regulation and control of the normal old heart. This study investigates, in man, the effect of age on the primary mechanism of cardiac control exerted by the sympathetic nervous system, and measures the response of hearts studied across a spectrum of ages (18-88) to induced work loads using noninvasive measures of heart size and performance provided by echocardiography.

FMS-6040
1-75

Project Description:

Objectives: Noninvasive techniques are employed in man to measure the function of the left ventricle while it is subjected to a known amount of stress. The rationale is that diseased or failing hearts may appear to function normally when not subjected to undue stress, but reveal abnormalities when subjected to even a moderate degree of stress. Previous animal experiments from this laboratory have demonstrated that the aged but otherwise healthy stressed heart has certain functional impairments. Comparable information in man is lacking. Utilizing modern techniques, it is possible to examine this question in man. By comparing the performance of normal (by standard criteria) young and aged hearts both unstressed and during the imposition of a controlled degree of stress, one is able to assess the degree of impairment in heart function due to the aging process.

Methods: Following last year's success with echocardiography, the current study utilizes the echocardiogram to measure left ventricular function during imposition of a stress. A predetermined increase in peripheral blood pressure is induced sequentially by isometric handgrip exercise or by infusion of an epinephrine-like drug (phenylephrine). Measurements are made before and after temporary blockade of the sympathetic nervous system achieved by infusing the beta adrenergic blocking drug propanolol. Electrocardiograms and blood pressure are recorded simultaneously with the echocardiogram. Left ventricular function is assessed by measuring changes in the diastolic and systolic dimensions and velocity of shortening of the endocardium during the various interventions.

Assessment of cardiac beta receptor-mediated and carotid presso-receptor-mediated control of heart rate is determined by measuring the changes in heart rate in response to infusions of isoproterenol, an epinephrine like compound that primarily stimulates the beta adrenergic receptors of the heart, and by phenylephrine, which increases blood pressure by stimulating the carotid receptors which in turn cause the heart to slow.

Major Findings: An expanded study of 105 subjects studied under resting conditions has verified the findings reported last year, with a decrease in mitral valve E-F slope (stiffer ventricle) and a larger aortic root diameter, both measured by echocardiography. The drug intervention study will require more subjects, although of the 19 studied to date ranging from 26 to 79 years, the older subjects appear to have a decreased sensitivity to adrenergic transmitters.

Significance to Biomedical Research and the Program of the Institute: Information concerning the functional impairment, if

any, of the aged but otherwise normal, healthy human heart as well as the sensitivity of the regulatory mechanisms is critical to understanding one aspect of the aging process. Results of this study may enable one to (1) evaluate and make better recommendations to elderly persons regarding the advisability of specific exercise activities, (2) help predict those people who are likely to develop significant heart disease as they age, and (3) better understand the overall changes in cardiac regulation as an individual ages.

Proposed Course: The drug study will be continued. The manuscript is in preparation for the baseline echocardiographic study of aging.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00011-04 CPB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Hormones, hormone receptors, and aging I. Aging and hormone-sensitive adenylate cyclase.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. S. Katz	Clinical Associate	CPB	NIA
	R. I. Gregerman	Chief, Endocrinology Section	CPB	NIA
Other:	None			

COOPERATING UNITS (if any)

Department of Surgery
Baltimore City Hospitals

LAB/BRANCH

Gerontology Research Center, Clinical Physiology Branch

SECTION

Endocrinology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	1.5	2.5

SUMMARY OF WORK (200 words or less - underline keywords)

These studies deal with influences of age on the biochemistry of hormone-sensitive adenylate cyclases in a variety of tissues. The purpose of these studies is to explore the mechanisms of age-related alterations of hormone responsiveness and biological membranes. The work utilizes preparations of materials from rats and human (liver, fat).

PHS-6040
(1-75)

Project Description:

Objectives: The mechanism of the initiation of the action of numerous hormones is activation of the cell (plasma) membrane-bound enzyme, adenylate cyclase. This hormone-responsive enzyme is responsible for the production of cyclic adenosine-3'-5' monophosphate (cAMP) from ATP. Numerous cell functions appear to be under control of cAMP. Aging is known to be accompanied by a variety of altered responses to hormones, but the role of adenylate cyclase in these events has only recently come under study. Our investigations are designed to provide new information in this area of hormone action in an attempt to understand the mechanisms by which aging affects hormone responsiveness (sensitivity). Since adenylate cyclase is a membrane-bound enzyme, such studies should also give insights into age-related alterations of cell membranes.

Methods Employed: Adenylate cyclase is determined by a labeled substrate assay in which ^{32}P-ATP is converted to cAMP by action of the enzyme. cAMP is isolated by a double-column technique. Use of ^{14}C and ^{3}H cAMP allow precise quantitation of the recovery in each experiment. Assays are performed on tissue homogenates, particulates, and purified cell membrane fractions isolated by standard methods of gradient centrifugation. For studies of fat cells, tissue samples are digested with collagenase to yield isolated cells prior to preparation of cell membranes. The cells are counted and sized (after fixation in OsO_4) in a Coulter apparatus.

Major Findings: 1. Adenylate cyclases (AC) of liver during maturation and aging. A study of age-related changes of epinephrine and glucagon sensitive AC has been completed and submitted for publication. Glucagon-sensitive AC increases about 10% in old female rats, but does not change in males. However, epinephrine-sensitive AC increases 100% (2-fold) in old animals. No significant difference is seen between 3 and 12 mo animals. The increase of activity is seen in association with decreased stability of AC in homogenates from old animals. This finding is in agreement with recent reports of increased enzyme activity associated with decreased stability for 3 cell (plasma) membrane bound ATPases from aging rat liver. For the latter enzymes the change has been related to altered phospholipid content of the membranes. Phospholipids are also known to be involved in the activity of AC. These findings suggest that age-related alterations of lipid metabolism of liver membranes may be responsible for the changes of hormone sensitive AC we have seen. Work by others with the ATPases suggests that some of the age-related change is reversible with dietary manipulation.

Preliminary studies of hormone-sensitive AC during early life show marked decreases of the enzyme at about 3 weeks of age. These changes appear to be related to the process of weaning, since we have now shown that delay of weaning prevents the sudden decrease. These preliminary results, taken with our earlier observations on the effects of dietary restriction and those of others mentioned above, all suggest that nutritional factors exert a major control of AC, perhaps by affecting cell membrane lipids.

2. Characterization of adenylate cyclase from human fat cells. The AC of human fat has not been previously studied. In anticipation of studies of aging in man we sought to characterize the enzyme from human material. A major difference between the well characterized AC of rat fat and AC from man was the absolute requirement of the latter for a guanine nucleotide activator. Although GTP was not effective under our assay conditions, and was actually inhibitory, epinephrine responsiveness was clearly evident when the GTP analog, GMP-PNP was added. In the absence of the analog epinephrine was without effect. This suggested a vital role for a guanine nucleotide and that under certain experimental conditions one might see enhancement by GTP itself. Preliminary experiments suggest that this is the case. The major controlling factors appear to be critical temperatures and, possibly, pH. The problem is under further study. In the meantime, fat specimens from some 50 human subjects have been assayed. AC levels and hormone responsiveness will soon be analyzed in terms of age, sex, clinical status, etc.

3. Anion activation of adenylate cyclase. Further aspects of the effects of anions on AC activity in rat and human fat have been studied. Some of the effect of Mg^{2+} at high levels has been attributed by others to the action of the cation. Our results indicate that the Cl^- anion is actually responsible. Other studies reveal complex effects of anions depending on the nature of the preparation (membrane vs. homogenate) and the concentration ranges of anions. At very low levels of anions, hormone sensitivity is enhanced. At high levels of anions, basal activity is increased. These effects are, to some extent, probes of the membranes to which AC is bound. Accordingly, we are exploring the complex interactions between guanine nucleotides, anions and aging in attempting to define effects of age on membrane structure and function.

4. Cytosol factor in the activity of epinephrine-sensitive adenylate cyclase. Attempts by ourselves and others to obtain purified cell membranes which retain epinephrine-responsiveness have long been unsuccessful. This problem frustrates any attempt to explore the effect of aging in enhancing cell epinephrine-sensitive AC. However, we have now succeeded in obtaining hormone-sensitive cell membranes. These studies suggest that, in addition to GTP and anions, a soluble cytosol factor may be involved. Efforts are now underway to isolate and define this material further.

Significance to Biological Research and the Program of the Institute: These studies are defining a key control mechanism for influences of age on hormone responsiveness. Furthermore, they are providing new insights into age related changes of membrane function.

Proposed Course of the Project: Current efforts involve further definition of the factors regulating adenylate cyclase responsiveness in human fat and their interrelationship with possible influences of aging. Studies

with isolated liver membranes are underway in order to explore influences of aging on adenylate cyclase at the membrane level. The importance of membrane lipids will be investigated, as will the nature of the cytosol factor for preservation of epinephrine responsiveness.

Publications:

Cooper, B., Partilla, J. S., and Gregerman, R. I.: Adenylate cyclase of human fat cells. Expression of epinephrine-sensitive activation revealed by 5'-guanylyl-imidodiphosphate. Journal of Clinical Investigation 56, 1350-1353, 1975.

Cooper, B. and Gregerman, R. I.: Hormone-sensitive fat cell adenylate cyclase in the rat. Influences of growth, cell size, and aging. Journal of Clinical Investigation 57, 161-168, 1976.

Cooper, B., Partilla, J. S., and Gregerman, R. I.: Human fat cell adenylate cyclase: Enzyme characterization and guanine nucleotide effects on epinephrine responsiveness in cell membranes. Biochimica et Biophysica Acta, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00012-04 CPB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Hormones, hormone receptors and aging. II. Aging and hormone responsiveness.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. S. Roth	Staff Fellow	CPB NIA
OTHER:	J. N. Livingston	Asst. Prof. Med.	Johns Hopkins
	R. J. Gregerman	Chief, Endocrinology Section	CPB NIA

COOPERATING UNITS (if any)
School of Medicine, Johns Hopkins University, Baltimore, Maryland.

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Endocrinology Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.0	0.2

SUMMARY OF WORK (200 words or less - underline keywords)

This project is mainly involved in relating age, the intracellular and cell surface receptors for hormones and the biological responsiveness of hormone-sensitive tissues.

PHS-6040
(1-75)

Project Description:

Objectives: The physiological and biochemical responses to hormones are altered during aging. Decreases in hormone receptor concentrations during aging seem to be at least partially responsible for such changes in responsiveness. The present study seeks to elucidate the cellular, molecular and neuroendocrine mechanisms by which receptor and corresponding responsiveness changes occur during senescence.

Methods Employed: Whole animals, isolated tissues, and defined cell populations in short term culture are used. Hormone receptors, located either on the cell surface or intracellularly, are studied qualitatively and quantitatively by measuring binding of labeled steroids, catecholamines and other hormones to tissues, cells and subcellular fractions. Hormonal control of various cellular metabolic process such as nutrient transport and utilization are measured by standardized techniques. Macromolecular biosynthetic processes are also assessed.

Major Findings: 1) Preliminary studies reported last year suggested that loss of glucocorticoid receptors form adipocytes might account for reduced responsiveness to these hormones during aging. An extension of these observations has been now completed and can be summarized here. Exposure of adipocytes to glucocorticoids for brief periods results in inhibition of glucose transport and metabolism. Maximal inhibition of glucose oxidation was found to decrease from 45 to 23 to 6% in young (2-3 mo), mature (12-13 mo), and senescent (24-26 mo) rat adipocytes, respectively. Percent values reflect absolute reductions since basal levels of glucose oxidation per cell are constant at all ages in both strains. Adipocytes of CD strain rats continue to increase in size throughout their lifespan, while cell size remains constant during the latter 80% of the Wistar adipocyte lifespan. Thus, cellular age, rather than simply cell size, is associated with these changes.

Concentrations as well as absolute numbers of presumptive glucocorticoid receptors (intracellular) per cell are progressively reduced during maturation and aging of adipocytes in both rat strains. Glucocorticoid effects require about 2 hours and can be blocked by various antimetabolites during this period. Moreover, when binding of glucocorticoids to adipocyte receptors is blocked by steroid analogs, no inhibition of glucose oxidation occurs. Thus, binding to receptors appears to be required to elicit this response. The gradual loss of glucocorticoid receptors from adipocytes during maturation and aging seems closely related to progressively decreased glucocorticoid responsiveness. A manuscript describing these findings has been prepared and submitted for publication.

2) Myocardial responsiveness to catecholamines has previously been shown to decrease during senescence. Since these hormones act through β-adrenergic receptors, it was of interest to examine the concentration of such receptors during aging. The currently employed alprenolol binding assay for such receptors was modified for use with mature and aged rat hearts. Time-courses of binding, stereoisomer specificity, affinity

and the concentration of receptors in mature rats were found to resemble very closely values published by others for dog hearts. A comparison of mature (6-12 mo) and senescent (24-26 mo) hearts revealed a 30-50% reduction in receptor concentration per unit microsomal protein but no change in binding affinity.

3) Studies in this laboratory over the past 2 years using defined post-mitotic cell populations (adipocytes and neurons) have shown that glucocorticoid receptors are actually lost from target cells during aging as opposed to mere loss of target cells from complex tissues. Thus, loss of receptors (and corresponding responsiveness) appears to take place through a "molecular" mechanism. Short term culture systems of neurons and adipocytes obtained from rats of different ages have therefore been established to study the effects of age on receptor synthesis and degradation. In synthesis studies, receptor proteins are being labeled by exposure of cells to radioactive amino acids for varying periods of time. Short "pulse" exposures followed by "chases" of unlabeled amino acids are used for studies of receptor turnover. Labeled receptors are selectively removed from other cellular proteins by affinity chromatography with dexamethasone coupled to Sepharose beads and quantitated by scintillation counting.

Significance to Biomedical Research and the Program of the Institute: A number of physiological and biochemical responses to hormones have been found to change with age in a manner which seems to be characteristic of a generalized altered ability to respond to various stimuli during senescence. Elucidation of the mechanisms underlying such altered responsiveness is of obvious interest and importance for understanding this aspect of aging.

The present study has focused primarily on changes in hormone receptors. The role of receptors in regulating hormone action has recently been the subject of intensive examination. Binding of hormones to receptors is the initial step in eliciting most hormonal responses, and in many systems responsiveness is dependent upon the amount of binding. The present project has added another dimension, namely the biology of aging, to hormone receptor theory and technology.

Proposed Course of the Project: With a few refinements, the glucocorticoid receptor synthesis and degradation systems recently developed in this laboratory should be ready for use. Studies will then attempt to determine whether decreased rates of synthesis and/or increased rates of degradation are responsible for decreased levels of receptors in aged cells.

Studies of β-adrenergic receptors in heart have to date expressed receptor levels per unit microsomal protein. It is possible that age differences in the recovery and composition of this somewhat heterogeneous fraction could account for some of the apparent aging loss of receptors. Thus, work in collaboration with the Cardiovascular Section will seek selectively to label radiochemically the cell membrane (which contains the β-adrenergic receptors) so that recovery of this particular fraction can be quantitated for different aged hearts. In this way receptor amounts per unit cell membrane are per whole heart can be determined. More reliable correlations between receptor levels and heart responsiveness will thus be possible. Ultimately it is hoped to understand the general mechanisms responsible for altered hormone

binding and responsiveness during aging. This may entail eventual purification of receptors for structural, functional and immunochemical measurements, as well as elucidation and manipulation of regulatory processes which control receptor quantity and characteristics.

Publications: Roth, G. S.: Age Related Changes in Glucocorticoid Binding by Rat Splenic Leukocytes: Possible Cause of Altered Adaptive Responsiveness, in Biology of Aging and Development (G.J. Thorbecke, ed.) Plenum Press, N.Y. 1975, pp. 315-319.

Roth, G. S.: Reduced glucocorticoid binding site concentration in cortical neuronal perikarya from senescent rats. Brain Research, in press.

Roth, G. S. Changes in hormone binding and responsiveness in target cells and tissues during aging, in Explorations in Aging, (V. J. Cristafalo, J. Roberts and R. C. Adelman, eds) Plenum Press, New York, 1975, 195-208.

Roth, G. S., Altered hormone binding and responsiveness during aging. Proceedings of the 10th International Congress of Gerontology Abstracts 1975, 44-45.

Roth, G. S., Reduced glucocorticoid responsiveness and receptor concentration in splenic leukocytes of senescent rats. Biochimica et Biophysica Acta, 1975, 399 145-156.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00013-02 CPB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Hormones, hormone receptors, and aging. III. Aging and the human male reproductive system.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. M. Harman	Senior Investigator	CPB	NIA
OTHERE:	C. E. Martin	Senior Investigator	CPB	NIA
	R. I. Gregerman	Chief, Endocrinology Section	CPB	NIA

COOPERATING UNITS (if any)
None

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Endocrinology and Human Performance Sections

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.0	0.2

SUMMARY OF WORK (200 words or less - underline keywords)

This project attempts to define influences of age on the male reporductive system, especially the production of male hormones (testosterone, dihydrotestosterone), the trophic hormones, LH and FSH, and the latters' responses to LH-RH. Correlations between hormone levels, hormone binding to plasma proteins and the interrelations of these factors with aging are being made in the longitudinal study subjects.

PHS-6040
(1-75)

Objectives:

A. Background- Sex hormones are important control mechanisms for development and function throughout the life span. The hypothalamus, an area at the base of the brain, regulates sexual function by secreting the hormone LHRH into the blood supply of the pituitary gland. LHRH stimulates the pituitary to release the gonadotrophins FSH and LH, which control gonadal function. In the male LH acts to promote production of the male sex hormone, testosterone, by secretory (Leydig) cells, while FSH acts together with testosterone to cause sperm production in the testis tubules. Testosterone, in addition, is responsible for developing and maintaining male secondary sex characteristics (body and facial hair, muscle mass) and plays an important role in sex drive. Testosterone also "feeds back" on the hypothalamus and pituitary to regulate secretion of the gonadotrophins.

B. Current knowledge- Relative to what is known concerning the hormonal events of early development, puberty, and function of the reproductive system during adulthood, the nature of the physiologic events surrounding senescence of this system have not yet been made clear. Several studies have shown that there is considerable loss of Leydig cell function beginning in men in their 50's. Circulating blood levels of total and especially "free" (non-protein bound) testosterone decreases along with an increase in circulating LH and FSH. These findings suggest decreased gonadal function as the primary event in producing decreased testosterone secretion with advancing age. Pituitary function appears to be preserved, and the response of elderly men to LHRH injection is reported to be normal. One group reported decreased testicular volume and decreased facial, pubic, and axillary hair in their older subjects. To date, however, none have attempted to correlate individual testosterone levels with changes in secondary sex characteristics, libido, onset of prostatism, or development of cardiovascular disease (long known to be more frequent in males). Studies of seminiferous tubular function in terms of quantity and quality of sperm production are also lacking. All available studies suffer from the limitation of being cross-sectional rather than longitudinal. If reproductive system functions (such as plasma testosterone) influence longevity, the population studied might become progressively skewed with advancing age.

C. Present study- Objectives are (1) to provide information on gonadal function in the aging human male with regard to gonadotrophin secretion and pituitary gonadotrophin reserve, testosterone production and testicular responsiveness to gonadotrophins, and semen production (2) to elucidate the relationships between pituitary, Leydig cell, and seminiferous tubular function in the aging male, with the object of determining the site (or sites) of failure of the reproductive system and (3) to correlate behavioral and health variables such as libido and prostate disease with levels of endocrine function in the aging human male.

Methods employed: (1) Plasma gonadotrophins are being assayed using a double antibody radioimmunoassay (2) Plasma testosterone, dihydrotestosterone, and estradiol are measured using a charcoal type radiommunoassay (3) Assay results are analyzed by a computerized method. (4) Semen analysis is performed using standard techniques for determining number and quality of sperm. (5) The free fraction of testosterone in plasma samples is estimated using DEAE cellulose "mini-columns". (6) Clinical samples obtained in two ways: (a) Longitudinal Study subjects are given endocrine stimulation tests with multiple serum samples taken at intervals, providing both baseline measurements and data regarding ability of the system to respond when stimulated. These tests are made with LHRH for pituitary gonadotrophin reserve and hCG for Leydig cell secretory capacity. In addition, a series of semen samples are being collected from those subjects able to cooperate. Libido is estimated from interview. (b) Freeze dried plasma samples, taken from the same longitudinal subjects in past years, will be analyzed in order to compare gonadotrophin and androgen levels with libido scores previously obtained and with other health parameters.

Major Findings: In the past 6 months methodological problems have been at least partially resolved and work with subjects was started. Only 21 men have volunteered for the study out of 65 eligible subjects invited to participate, a disappointing proportion. Nonetheless, preliminary inspection of the data suggests that there is no systematic change of sperm count or testis size with age, but many more subjects must be studied before any conclusions can be drawn. At the present rate, at least 18 additional months will be required to collect a minimally adequate number of subjects.

Assay for testosterone and protein binding of testosterone can now be done reliably in our laboratory. Assays for dihydrotesterone, estradiol, LH, and FSH are still under development. One delaying factor is the small volumes of stored plasma samples requiring development of micro-techniques.

Major technical problems have been the development of simple methods for estimation of plasma "free" (non-protein bound) as opposed to total testosterone and for the assay of dihydrotestosterone and estrogens with the small volumes of plasma available. The problem of "free" testosterone appears to have been solved by development of a new DEAE (ion-exchange) batch elution mini-column method. This technique provides a more convenient estimate of "free" testosterone than does the conventional method of equilibrium dialysis. Studies in our laboratory have shown a high correlation between the results of the column method and that of dialysis ($r = 0.98$). A manuscript describing the method has been prepared for publication.

The problem of measuring dihydrotestosterone in plasma is also nearly solved. This testosterone derivative is the active metabolite of testosterone and may change with aging. Problems of measurement relate

to the small quantity present in plasma and lack of antibody specific enough to avoid significant cross reactivity with testosterone. At present our experience suggests that a solution can be found in a fairly simple separation technique for dihydrotestosterone and testosterone on mini-columns of the partition type (Celite) followed by immunoassay. An even simpler column adsorption method is under development and shows promise in separating dihydrotestosterone, testosterone and estrogens prior to their immunoassay.

Significance to Biomedical Research and the Program of the Institute: Quantitative information on the decrease in male reproductive function with age which correlates hormonal, psychological, and other variables is sorely lacking. What proportion, if any, of the increased incidence of impotence and decreased sex drive in aging men is endocrinologic, and therefore potentially reversible, is not known. Data regarding the interactions of age, reproductive physiology and sexual behavior will add a new and useful parameter to the GRC longitudinal study, and may suggest both specific therapy for sexual dysfunction and new avenues of investigation into the nature of the aging process.

Proposed Course: The investigators will continue to invite subject participation in the study as outlined, attempting to increase volunteer participation. Methodologic development will continue. The determination of plasma estradiol (female hormone) has been added to the study. Recent investigations in the field have suggested that older men have increased secretion of such hormones. Such an event could be responsible for an increase of the plasma testosterone binding protein which produces a decrease in the amount of "free" (available) plasma testosterone.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00023-01 CPB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Hormones, hormone receptors and Aging, IV. Aging and Leydig cell function.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. M. Harman	Sr. Invest.	CPB	NIA
OTHER:	G. S. Roth	Staff Fellow	CPB	NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Gerontology Research Center, Clinical Physiology Branch

SECTION

Endocrinology Section

INSTITUTE AND LOCATION

NIA, GRC, Baltimore, Md. 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

SUMMARY OF WORK (200 words or less - underline keywords)

This project is an attempt to develop an animal model system for studying the effects of aging on Leydig Cell function (testosterone production) in the male. The blood levels of testosterone and the responses to stimulation with chorionic gonadotropin are under investigation in various strains of rat.

PHS-6040
(1-75)

Project Description:

Objectives: The testicular Leydig cell is the source of the male hormones, principally testosterone, which produce masculine secondary sex characteristics and maintain male sex drive both in man and animals. It is apparent that in aging humans these cells produce less male hormone in response to gonadotrophin stimulation. A similar situation has been described for the rat by several investigators.

The testicular secretion of testosterone is under the control of the pituitary gonadotrophin, LH. In addition to stimulating increased secretion of testosterone by pre-existing Leydig cells, LH recruits undifferentiated interstitial cells located in the tissues of the testis and causes them to become steroid secreting cells. The mechanism of LH stimulation involves its binding to specific receptors on the outer membrane of the cell followed by activation of tne membrane-bound enzyme, adenylate cyclase, which in turn catalyzes the conversion of ATP to cyclic AMP (cAMP). cAmp accumulation is responsible for multiple effects within the cell, and leads to increased synthesis and/or activation of enzymes which in turn convert cholesterol to sex steroid hormones. Our present goal is to define an animal model for the aging Leydig cell and to investigate the biochemical and cytological basis of age-related defects of this cell population.

Methods employed: Rats of various ages are subjected to Leydig cell stimulation with injections of hCG (an available gonadotrophin similar in action to LH) using 2 different protocols: a long term (3 day) series of injections to measure the ability of the animal to differentiate Leydig cells from the inactive interstitial cell population, and a short period of stimulation (3 hrs) designed to measure responsiveness of Leydig cells already present. Blood samples taken at various intervals are analyzed for testosterone by radioimumunoassay.

Rats will also be studied to determine the differences in the rate at which young and old animals metabolize testosterone, so that differing plasma levels can be understood in terms of the balance between formation and destruction of the hormone. This determination will be made by turnover measurements of radioactive testosterone after intravenous injection of isotopically labeled hormone.

When a suitable model of aged Leydig cell dysfunction has been established with intact animals, Leydig cells will be isolated in vitro. We hope to examine eventually the synthesis of testosterone, the number and character of hCG receptors, adenylate cyclase activation and the activity of enzymes required for sex steroid synthesis. In addition, histological sections of rat testis will be examined to quantitate and characterize the Leydig cell population in old and young rats before and after stimulation.

Major Findings: To date both long and short term hCG stimulation experiments in vivo have revealed rather large variability in the responsiveness of serum testosterone to hCG. Accordingly, a fairly large number of animals have had to be used in order to permit statistically valid conclusions. In the Sprague-Dawley strain of rat we have been using, old (24 mo) rats have (unstimulated) plasma testosterone levels only half those found in younger rats (12 mos), but hCG stimulation of older animals in either long or short term experiments produces increases of plasma testosterone equal to or slightly greater than those of young rats. This finding has made it obvious that the model does not correspond to that reported in humans or other strains of the rat.

Significance to Biomedical Research and the Program of the Institute: This investigation hopes to define the cytological and biochemical defects in a population of hormone responsive cells showing decreased function with age. Such a study should help to elucidate the nature of tissue and cellular aging.

Proposed Course: Since investigations to date have not succeeded in defining a model for Leydig cell aging, it will be necessary to investigate other strains of rat and possibly other species in attempts to find animals whose aging process more closely corresponds to that reported for man.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00014-06 CPB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

The Biochemistry of renin and renin substrate.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. J. Chou	Visiting Fellow	CPB	NIA
OTHER:	R. I. Gregerman	Chief, Endocrinology Section	CPB	NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Endocrinology Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	1.3	0.1

SUMMARY OF WORK (200 words or less - underline keywords)

This project explores the biochemistry of renin and renin-substrate, proteins involved in hypertension. The substrate is being purified and labeled in order to develop a new assay for renin. Other studies inclued chemical modification of the substrate and synthesis of polymeric substrates and renin inhibitors.

PHS-6040
(7-7)

Project Description:

Objectives: The secretion of renin, an acid proteinase produced by the juxtaglomerular cells of the kidney is under the control of a variety of factors. In the circulation the enzyme acts on a plasma glycoprotein to release angiotensin I, a decapeptide which is then activated by C terminal cleavage to a smaller octapeptide, angiotensin II, in the lung and other tissues. Angiotensin II in turn influences the secretion of aldosterone, principal mineralocorticoid of the adrenal, and has other direct effects on the cardiovascular and central nervous system. In certain pathologic conditions renin has a direct role in the pathogenesis of hypertensive disease, as it does in renovascular stenosis. Renin is also indirectly implicated in other forms of hypertension. The secretion of renin (and aldosterone) is markedly influenced by aging in man, and hypertension is an age-dependent disease.

Because of these latter considerations, our laboratory has had an interest in renin and angiotensin, especially in development of new techniques for measurement of the enzyme and its peptide products, in the biochemistry of the enzyme and its substrate, and the relevance of the renin-angiotensin-aldosterone system to normal and pathologic aging.

Methods Employed: Renin has been assayed by our previously published polymeric substrate assay. Renin substrate has been partially purified from porcine plasma. Further purification involves DEAE cellulose column chromatography and columns of Con A-Sepharose, and Sephadex G-100. Other techniques are standard analytical procedures of peptide chemistry (amino acid analysis, high voltage electrophoresis, etc,). Localization of substrate is made by radioimmunoassay of angiotensin I generated with added renin.

Major Findings: I. The Chemical Nature of Renin's Natural Substrate. An ester linkage between the hydroxyl group of serine of the N-Terminal tetradecapeptide and the carboxyl group of either arginine or lysine in the rest of the protein renin substrate has been postulated. If a unique ester linkage existed, we had to know something about this in order to be able to fashion a proper substrate for assay of the renin in human plasma. Such a linkage would be unique, to say the least, and could be important in determining the specificity of the renin-renin substrate reaction.

Using partially purified porcine renin substrate, we obtained apparent confirmation of the result of others. Treatment with alkali under specified conditions yielded a product which appeared to be the tetradecapeptide N-terminal fragment. This finding suggested that the C-terminal serine of the tetradecapeptide was involved in a unique linkage to the protein, viz., an ester bond. More complete examination of this product, however, showed that it was in fact the N-terminal tridecapeptide (des-Ser). We then used two milder esterolytic reagents (1 M hydroxylamine,

pH 9.5, 40°, 1.5 hrs.; 1 M hydrazine, pH 9, 40°, 5 hrs.) and again isolated the same tridecapeptide. This finding indicates that the peptide bond between serine and tyrosine is alkali labile. Lithium borohydride in tetrahydrofuran quantitatively cleaves ester bonds while peptide bonds remain intact. However, with this reagent we did not isolate any peptide product. We concluded that the tetradecapeptide renin substrate is linked to the rest of the protein renin substrate by an alkali labile seryl peptide bond rather than an ester linkage. This work is being prepared for publication.

II. The Specificity of Renin Substrate and Development of a Labeled Substrate for Renin: Renin appears to possess unparalleled and unique specificity among proteinases. However, this apparent specificity may be related to the chemistry of natural glycoprotein rather than to the proteinase. Protein renin substrate is hydrolyzable by renin but not by pseudorenin. The introduction of a label into the angiotensin I sequence portion of the protein should allow the retention of reaction specificity for renin and also provide an alternative method for assay of the angiotensin analog generated. We are exploring this possibility with the hope of eventually being able to produce a labeled protein renin substrate which will prove practical for rapid assay of renin in human plasma.

Renin substrate has in the past been partially purified from hog plasma and separated into three major (A, B, and C) and two minor forms (D and E). Differences exist in the sialic acid, glucosamine and natural hexose contents which may account for different physical properties. Since only two forms of renin substrate have been purified and in only small amounts, there exists a need for a simpler, more specific isolation method to allow large scale preparation of purified protein renin substrate prior to labeling attempts or other biochemical experiments.

Concanavalin A (Con A), a glycoprotein obtained from jack beans, forms complexes with certain polysaccharides and glycoproteins. Studies of its interaction with polysaccharides have revealed that it binds to molecules containing α-D-gluco-pyransoyl, α-D-mannopyransol, α-D-glucosaminyl or sterically related sugar residues and that the resultant complexes are dissociated by methyl-α-D-manno-pyranoside and methyl-α-D glucopyranoside. Considering the fact that Con A has the property of binding specific sugars, a number of applications of Con A have emerged utilizing the unique property. For example, Con A-Sepharose affinity chromatography has been shown useful in purification of several glycoproteins.

Preliminary studies in this laboratory show that the renin substrates behave differently in their ability to bind Con A. This indicates that sugar moieties in the protein renin substrates interacting with Con A have α- and β- anomeric forms. This preliminary work suggests that Con A-Sepharose affinity chromatography will be useful not only in purification of the protein renin substrate but also in studying the nature of the specificity involved in the interaction between Con A and renin substrate.

Recently, the preparation of ^{125}I-labeled sheep renin substrate for assay of renin has been reported. However, the method is not ideal,

possibly because the sheep renin substrate is only 25% pure and labeled by chloramine-T. As a result a variety of the tyrosine groups in the molecule are labeled. The material's specific activity is too low and the "blank" too high to be useful for the determination of renin concentrations in human plasma. We propose to label purified porcine renin substrate with a new technique for labeling proteins at the N-terminal. We should obtain a high specific activity material suitable for determinaton of the low renin concentrations in human plasma. Interference by pseudorenin will be no problem; this assay will be specific for renin. Moreover, simple solvent extraction or absorption isolation of the labeled peptide product should allow an extremely easy assay procedure. If successful, this approach should provide a valuable alternative to present immunoassay procedures.

III. Polymeric Inhibitors of Renin: Previously, we synthesized a dextran-ethylenediamine conjugate. This polymer conjugate has a free amino group and can be used to couple to the C-terminal of pepstatin, or other peptide inhibitors of renin. However, we found that during the activation of dextran by adding cyanogen bromide, most of the dextran became insoluble. For this reason the coupling of pepstatin to the polymer conjugate was unsatisfactory. Recently, a modification of this method, employing low concentrations of cyanogen bromide to avoid irreversible precipitation of the dextran during the activation reactio, has enabled us to synthesize a soluble dextran-ethylene-diamine conjugate. It should not be difficult to couple this material to pepstatin. Once this pepstatin complex has been made and characterized it will be used for inhibition testing with renin.

Significance to Bio-medical Research and the Program of the Institute: Our studies have defined the chemical relationship of renin to other proteinases. Present work may allow an explanation of renin's specificity and the development of new classes of renin inhibitors and labeled protein renin substrates. This information may be useful eventually for practical applications to problems related to the diagnosis and treatment of hypertensive diseases.

Proposed Course of the Project: Our immediate objective is purification of protein renin substrate for labeling. Once the labeled protein renin substrate has been made, kinetic studies will be undertaken. Testing of the pepstatin-polymeric conjugate will proceed.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00015-18 CPB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

The Baltimore Longitudinal Study of Human Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	N. W. Shock	Chief, Gerontology Research Center	NIA
	R. Andres	Chief, Clinical Physiology Branch	CPB NIA
	A. H. Norris	Chief, Human Performance Section	CPB NIA
OTHER:	D. Arenberg	Chief, Learning & Problem Solving Sec.	LBS NIA
	B. D. Bricker	Computer Specialist	CPB NIA
	M. Butler	Dietitian	CPB NIA
	B. T. Engel	Chief, Laboratory of Behavioral Sciences	LBS NIA
	C. E. Martin	Sociologist	CPB NIA
	C. C. Plato	Geneticist	CPB NIA
	J. D. Tobin	Medical Officer	CPB NIA
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	B. H. Cohen*	Professor of Epidemiology	
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COOPERATING UNITS (if any)

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LAB/BRANCH

Clinical Physiology Branch

SECTION

Human Performance Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.50	1.85	3.65

SUMMARY OF WORK (200 words or less - underline keywords)

The Baltimore Longitudinal Study serves as a resource for scientists working in the field of Gerontology. It provides a well-described group of men between 20 and 96 years of age for studies of the mechanisms of human aging. Projects in physiology, biochemistry, psychology, nutrition, pharmacology, endocrinology, sociology, and genetics, have been carried out or are in progress.

Objectives: The Baltimore Longitudinal Study provides a well described group of subjects as a resource in support of a wide variety of scientific investigations in gerontology and other disciplines. While long-term planning is encouraged, important studies of shorter duration have also been undertaken. The long-term general goals of the project are to: (1) secure replicate measures of physiological, pathological, biochemical and psychological variables on longitudinal study participants at specified intervals; (2) summarize and compare the results of testing in relation to age according to cross-sectional and longitudinal formats; (3) identify characteristics of individual participants which may be related to changes of function over time and to age at death; and, (4) determine whether the data obtained support one or another theory of the mechanisms responsible for age-related functional decrements.

Methods Employed: The Sample: Study participants are male volunteers recruited by other participants in the program. Recruits agree to return to GRC in Baltimore for 2-1/2 days of testing every 12 months (age 70 and over), 18 months (age 60-69) or 24 months (under age 60) for an indeterminate period. At entry into the program, 87% of subjects reported at least some college, 87% were identified with professional, technical or managerial occupations, 90% were presently married, 83% described themselves as financially comfortable or better, and of the group who returned for the fifth visit, 90% had rated their health as good or excellent on both first and fifth visits.

Data Management: Medical records and test results are maintained in written form in the laboratory and transferred to a data retreival and analysis system by keypunching on tabulation cards or by recording the test results directly on punched paper tape or magnetic tape. Data are maintained and used in ways which protect the privacy of participants. Sensitive material is specially encoded. Individual scientists review, evaluate and summarize the data for scientific reporting.

Major Findings: This section includes information about maintenance of the sample, and planning and operation of the overall study. Research findings are included in reports of investigators who use longitudinal studies participants as subjects in their work. On March 31, 1976, 1050 participants had been tested during one or more visits to the GRC. There was a total of 5547 participant visits since 1958. Seven hundred and sixty-one subjects had been tested 3 or more times, 555 had been tested 5 or more times, 283 at least 8 times, 134 at least 10 times and 47 had been tested 12 or more times. Since the beginning of the study, 147 participants have died and 250 have withdrawn from the study, leaving a total active sample of 653 men.

Since its inception in 1958, the Baltimore Longitudial Study of Aging has relied on a study population comprised of non-institutionalized male volunteers of highly diverse age. The sample is self-selected, with some 90 percent having been recommended for membership by a friend, relative or acquaintance already in the program. This method of recruit-

ment generated a sample which has many features of an elite segment of the general population.

Over the years, as participants were lost from the study through death, disability or withdrawal, additional subjects were admitted to observation to replace these losses and to further expand sample size to a current level of about 650 subjects. Since various uncontrolled factors have influenced participants both in their election to join this rather demanding study and their decision to discontinue participation, the question arises as to whether the overall character of the study population may have changed over the 16-year course of the project. To address this question, subjects who had appeared for testing in each two-year cycle between Cycle A (February, 1958 to June 30, 1961) and Cycle H (July 1, 1973 to June 30, 1975) were identified and their personal characteristics at initial visit determined for comparison over eight separate cycles.

Various criteria of comparison have been summarized with the maximum and minimum values obtained and the particular cycles where they occurred.
1. Age: Mean age at first visit. High 56.61 years (G); low 53.04 years (A). A trend is observed toward increasing age over the course of the study.
2. Health, self-report: Percent rating their health as good or excellent at first visit. High 94.4% (H); low 92.6% (D). Highly uniform over all cycles.
3. Education: Percent with a bachelor's or higher academic degree at first visit. High 79.7% (C); low 77.5% (E). Remarkably uniform over all cycles.
4. Occupational class: Percent identified with professional, techanical and managerial occupations. High 91.2% (A); low 84.4% (H). Some drift to the extent that subjects of lesser occupational status increased from 9 to 16% across cycles.
5. Economic status: Percent rating their situation as comfortable or better. High 84.3% (b); low 82.4% (H). Very uniform over cycles.
6. Marital Status: Percent married at first visit. High 91.9% (A,B,C); low 88.6% (H). Very constant percentages.
7. Religious affiliation: Percent Protestant at initial visit. High 78.9% (A); low 73.3% (H). Uniform over Cycles A to E, then some slight increase in proportion Catholic.
8. Activities and Attitudes Questionnaire: Mean score as indicator of social adjustment. High 69.75 (C); low 69.20 (G). Only slight fluctuation, no drift.
9. Cornell Medical Index: Mean score as indicator of symptomatology. High 14.15 (F); low 13.52 (H). No trend by Cycle.

Conclusion: The generally high socioeconomic, health and marital status characteristics of the study population are consistently maintained over the history of the study. Moreover, the high mean scores obtained on the Activities and Attitudes Questionnaire reflect the high level of social adjustment found among the study population, while the low scores for the Cornell Medical Index describe their relative freedom from

serious symptomatology. Neither measure is found to vary to a significant extent over the course of the program.

A longitudinal study of the increase in severity of osteoarthritis is reported in the project entitled, "Epidemiological Investigations of Osteoarthritis of the Hand."

A twelve-year follow-up of physiological responses to manual exercise is reported in the project entitled, "Age Changes in Human Performance."

Significance to Bio-Medical Research and the Program of the Institute: A major goal of the longitudinal program is a deeper understanding of age-related changes in the different organ systems, and their interrelationships. The relation of functional changes in an individual to age at death, age of onset of a disease, and other end points is important for understanding aging in humans and the impact of aging on society. The intensive study of multiple variables will also provide tests of risk-factor theories for specific age-related diseases.

Proposed Course: Data collection and analyses will be continued. Continued emphasis on automation of tests, data entry, and analyses should provide improved accuracy and efficiency. A major summary of all aspects of this program is in progress.

Publications:

Andres, R., Tobin, J. D., Norris, A. H., and Shock, N. W.: Quantification of the Rate of Physiological Aging in Man. In: Proceedings of the 10th International Congress of Gerontology, Vol. 1, Plenary Sessions, Symposia. The Congress, Jerusalem, Israel, June 1975, pp. 183-185.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00016-21 CPB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Age Changes in Human Performance

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. H. Norris	Chief, Human Performance Section	CPB NIA
OTHER:	F. Garfinkel	Clinical Associate	CPB NIA
	S. P. Tzankoff	Staff Fellow	CPB NIA
	N. W. Shock	Chief, Gerontology Research Center	NIA
	A. T. Welford*	Professor of Psychology	
	R. Fitzgerald**	Assoc. Prof. Environmental Medicine	
	D. G. Carroll***	Chief of Rehabilitation Medicine	

COOPERATING UNITS (if any)

***Baltimore City Hospitals
**The Johns Hopkins Medical Institutions, Baltimore

*Department of Psychology
University of Adelaide
Adelaide, South Australia

LAB/BRANCH
Clinical Physiology Branch

SECTION
Human Performance Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.50	2.30	4.20

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study the mechanisms and the limitations of a variety of physical activities in old and young individuals. Muscular activity ranges from brisk walking on an inclined treadmill to tapping between targets of various widths drawn on paper and separated by various distances. Exercise responses are measured for blood pressure, heart rate, pulmonary ventilation, carbon dioxide elimination, and oxygen uptake. The oxygen cost of exercise is measured and compared to the total amount of physical work performed to estimate the mechanical efficiency of the subjects' neuromuscular and psychomotor control systems. Responses of the pulmonary system are interpreted in terms of standard spirometry and dead space (residual volume) measurements as well as studies of respiratory control. Limitations on performance imposed by cerebrovascular, cardiovascular, and pulmonary disease are assessed. Reflex time, reaction time and speed and accuracy of movement are measured and compared with exercise responses.

Objectives: This project is designed to study the effects of aging on the physiological responses to and recovery from exercise--to describe age changes and to elucidate the mechanisms of these effects of aging. It is designed to identify underlying factors in the limitation of work performance and reduced mechanical efficiency in older people. For this purpose, detailed evaluation of pulmonary function and pulmonary response to stressful agents are carried out. Other factors such as the metabolic cost of limb movement and psychomotor control of limb movement are being studied.

An additional goal is to identify and explain the role of disease-altered physiological function in age-related limitation of work performance. Cerebrovascular, cardiovascular and pulmonary disease and functional measures such as blood pressure, reflex time, and reaction time will be considered.

Methods Employed: Measured amounts of physical work are administered to subjects of varying ages by means of a calibrated arm ergometer and quantitative mechanical analysis of limb movement. A treadmill is used to induce higher levels of work. Measurements of oxygen uptake, CO_2 elimination, pulmonary ventilation volume, heart rate, blood pressure, and electrocardiogram are made before, during and after standardized amounts of exercise. The functional capacities of the pulmonary system are evaluated. Alterations in respiratory function as a result of the stimulation of low oxygen and high oxygen and carbon dioxide in the inspired air are evaluated by pressure changes induced by occlusion of airflow ($P_{0.1}$).

Major Findings: RESPIRATORY CONTROL: Whitelaw et al. (Resp.Physiol., 13:181-199, 1975) have proposed using the pressure developed in the mouth 0.1 seconds into an occluded inspiraton, $P_{0.1}$, as a measure of central respiratory neuronal discharge. The measurement is made under no flow conditions and therefore, there should be no interference from the Hering-Brewer inflation reflex nor should there be any negative influences of an abnormal lung tissue compliance or resistence. There are at least two factors which could alter the interpretation that $P_{0.1}$ reflects only medullary center neuronal output: (1) changes in respiratory muscle length, as reflected by functional residual capacity (FRC), and (2) changes in the status of the contractile mechanism of the respiratory muscles.

The present work was aimed at determining: (1) if $P_{0.1}$ was a good indicator of central respiratory control; (2) what influence age had on this measurement; (3) if FRC changes in response to certain respiratory stimuli; and, (4) what contribution change in FRC made to the $P_{0.1}$ measurement.

Subjects ranged in age from 20 to 86 years. They were participants in the Baltimore Longitudinal Study and staff members of the Gerontology Research Center. The subjects abstained from food, drink and smoking for approximately 2 hours before being tested. They emptied their bladders immediately before the test. The test consisted of being seated in an air conditioned

volume displacement body plethysmograph, listening to music via headphones and breathing either room air, air with 4% CO_2 added, 11% O_2 balance N_2 or 100% O_2. The subject never knew which gas he was breathing. At fixed periods while breathing each gas mixture, measurements were made of heart rate, respiratory frequency and tidal volume, FRC, $P_{0.1}$ and alveolar CO_2 and O_2.

Analysis of a sample of the total data indicates that $P_{0.1}$ is very well correlated with ventilation with all gases given and at all age levels. The FRC was found to definitely increase when hypercapnic or hypoxic mixtures were breathed and definitely decrease when hyperoxic conditions were maintained. Although FRC definitely changed, there was only a very weak correlation overall with $P_{0.1}$. When partial correlations were done comparing $P_{0.1}$ with ventilation and FRC, FRC had almost no contribution at all. Dividing the subjects into age decades, it is found that there is a correlation of $P_{0.1}$ with FRC for those subjects < 30 years of age, but no correlation for older subjects. Since the majority of the subjects studied were > 30 years old, this probably accounts for the very weak overall correlation.

When looking at the data divided into young ($\leq$ 40), middle (41-59) and old age ($\geq$ 60), it is found that the old group tends to have higher values for FRC and $P_{0.1}$ but with far more scatter. But there is a significant increase in the percent change of $P_{0.1}$ when going from air to CO_2 breathing and from air to low O_2 breathing in the old group. This occurs despite the fact that the percent changes in the FRC's are about the same for each age group. This is depicted in the following comparison of % change $P_{0.1}$ and FRC from breathing air to breathing 100% O_2, 4% CO_2 and 11% O_2 in young, middle, and old age groups:

	< 40	41-59	> 60
% change in mean $P_{0.1}$			
100% O_2	-11.9	-19.2	-15.3
4% CO_2	+63.6	+59.6	+84.7
11% O_2	+26.3	+42.3	+48.3
% change in mean FRC			
100% O_2	-8.8	-11.6	-11.3
4% CO_2	+12.1	+11.9	+16.2
11% O_2	+12.1	+ 9.3	+15.5

Our results tend to confirm the findings of Whitelaw et al. that $P_{0.1}$ is a good indicator of central ventilatory control. The results also show that although changes in $P_{0.1}$ response increase with age and FRC also increases with age, there is no strong influence of FRC increases on the $P_{0.1}$ response. This is surprising as one would expect the "pre-stressed" muscle length to have a definite effect on the pressure generated by the

muscle. One possible explanation is that as the body ages and FRC increases, the thoracic muscles are working at the upper end of the length-tension curve and increases in length have only a very small influence on the force generated. It is possible that this increase (or decrease) in force is just too small to pick up with our techique of occlusion pressure measurement.

EXERCISE RESPONSES: Physical fitness has long been thought to impart protection against the development of coronary heart disease. The oxygen consumption of a subject performing maximal treadmill exercise--the maximal oxygen consumption--is an objective measure of physical fitness of individuals of varying ages and physical conditions. A poor performance on the treadmill test might be considered as a risk factor for coronary heart disease. In order to evaluate physical fitness as a risk factor, longitudinal study participants are being classified according to their performances on the treadmill test and will be followed to determine when and if they develop coronary heart disease or other cardiovascular disorders in the future. Those tested are free of cardiovascular disease as determined by physical examination, normal resting electrocardiogram, and review of medical history.

Subjects who met the strict medical criteria and gave informed consent to participate in the study walked on a motor driven treadmill at a constant speed of 5.6 km/h (3.5 m.p.h.). The incline of the treadmill was raised by 3% grade increments every two minutes until each subject reached his maximal work capacity. Oxygen consumption was measured at each work level while electrocardiographic monitoring was done continuously during the work and in the recovery. Venous blood samples were obtained at short intervals during the first few minutes of recovery for the determination of lactic acid concentration, an index of muscular work stress.

Treadmill exercise data on 56 men have been obtained to date. Of those, 51 were considered to give technically satisfactory values of maximal metabolic measurements. Analysis of the data by age decades revealed that the maximal values are in good agreement with those cited in the literature for healthy men. When compared with subjects averaging 25 years of age, the older men (64 years of age) showed lower mean values for maximal work capacity (-21.9%), maximal lung ventilation (-21.5%), maximal heart rate (-10.8%), and maximal oxygen consumption (-17.6%). Mean blood lactic acid values taken in the fifth minute of recovery were 27.1% lower for the old men than for the young.

Number in group	6	7	12	9	12
Mean Age (years)	25.8	35.2	44.1	53.1	64.5
Lactic Acid (mg%)	98.5	84.3	84.0	71.0	71.8

Blood samples for lactic acid taken at 3 and 7 minutes of recovery showed that although the younger men's concentrations had reached maximal values by 3 minutes of recovery, even the 40 year olds had lower values at 3 min. (65 mg%). This suggests that circulatory and/or diffusion changes in the

muscles may be delaying the movement of lactic acid into the blood in older subjects.

The data for a submaximal work rate (6% grade) were also examined. They revealed that the old men were less efficient in performing at this work rate. They required, on the average, 14.8% more lung ventilation and 5% greater oxygen consumption than the young men. The old men were, however, able to perform this work at a lower heart rate (-4.8%). This greater cardiac efficiency may be a result of selection since older men who have not yet developed heart abnormalities were compared with younger men who may develop abnormalities before they reach the age of the older men with whom they were compared.

We have conducted a 12-year follow-up of physiological responses to manual exercise in nine men who were from 27 through 71 years of age when they were first tested. The participants in this study performed submaximal rates of work which were about one-half of the maximum work rate they could achieve with an arm cranking exercise on a bicycle ergometer during a preliminary trial. The total amount of work performed was determined by the length of time the participants could maintain the prescribed work rate. Any subsequent part of the exercise task when work rate was declining from the predetermined level was added after making allowance for lower work rate. Responses to and recovery from the work task were monitored for ventilation volume, carbon dioxide production and oxygen uptake. These variables were also measured during a preliminary period immediately before the beginning of exercise while the subject was in a basal metabolic state as usually defined. Basal values from this preliminary period were used as a baseline for the computation of the extra oxygen (in excess of basal) which was associated with the work task. The ratio of the caloric equivalent of the total work performed to the caloric equivalent of the extra oxygen used by the subject was the net mechanical efficiency.

Mechanical efficiencies ranged from 8.0% to 14.4%. Each of the 5 participants who were 27 to 34 years of age at the time of the first test (young group) increased his mechanical efficiency between the first test and the follow-up tests. The average increase was 2.32 $\pm$ 0.99%. Each of the four participants who were 41 to 71 years of age at the time of the first test (older group) decreased his mechanical efficiency between the first test and the follow-up test. The average decrease was 3.73 $\pm$ 0.98%. The age of 40 years would seem to be rather early for the beginning of decline in the factors which contribute to the maintenance mechanical efficiency in man. We have previously shown, however, that one of these factors, coordination ability, began to decline at about age 40. In the earlier study, capacity for coordinated work was measured as the maximum power output which could be generated by the participant for a short burst of arm cranking exercise on the bicycle ergometer. Muscle strength which represented the anatomical capacity for performing work was maintained at young adult levels for participants into their sixties. Since cardiovascular and pulmonary limitations were ruled out because of the short

duration of the work task and tissue loss (strength decline) was not found earlier than age 65, reduction in maximum power generating capacity could be attributed to a decline in that complex set of control mechanisms we call coordination ability. This decline would seem to be at least a partial explanation of the mechanical efficiency findings of a shift away from a capacity to improve after age 40.

Significance to Bio-Medical Research and the Program of the Institute: Failure of control of breathing, whether acute or longer term, can have life threatening consequences. Understanding of the relationship between the central nervous system and lung can only assist in development of life saving techniques and life support systems and in the treatment of chronic respiratory disease.

The decline of the ability of some older people to perform their day-to-day activities and to engage in pursuits which contribute to the economic and social strength of our society represents a national loss. Identification of the physiological, medical and social correlates of high levels of physical strength and psycho-motor performance in middle and old age, as well as declines in these abilities, should lead to techniques designed to reduce the rate of decline in performance capacities with age.

Proposed Course: Measurements of muscle strength and maximum power generating ability during arm exercise will be continued. Cardiovascular, ventilatory and metabolic responses to standardized arm ergometer exercise and monitored treadmill exercise will be used to classify participants into fitness categories and to explore the age relationships of biochemical and metabolic responses to exercise. Measurements of lung volumes and uniformity of pulmonary ventilation will be made to characterize the respiratory competence of the longitudinal studies participants. Measurement of respiratory drive in relation to various stimuli will be evaluated in these participants.

Publications:

Fish, J.E., Rosenthal, R.R., Batra, G., Menkes, H., Summer, W., Permutt, S. and Norman, P.: Airway responses to methacholine in allergic and non-allergic subject. Am. Rev. of Resp. Dis. 113: 579-586, 1976.

Rowe, J.W., Andres, R., Tobin, J.D., Norris, A.H. and Shock, N.W.: The effect of age on creatinine clearance in men: A cross-sectional and longitudinal study. J. Geront. 31:155-163, 1976.

Rowe, J. W., Andres, R., Tobin, J. D., Norris, A. H., and Shock, N.W.: Age-adjusted standards for creatinine clearance. Annals of Internal Medicine 84: 567-569, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00017-18 CPB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Age Relationships of Body Composition, Nutrition and Physical Activity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A.H. Norris	Chief, Human Performance Section	CPB NIA
	R. Andres	Chief, Clinical Physiology Branch	CPB NIA
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	R. Aamodt	Chief, Whole Body Counter Section	NM CC
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	S.M. Garn **	Professor of Anthropology & Professor of Human Growth & Development	

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.45	1.05	3.40

SUMMARY OF WORK (200 words or less - underline keywords)

This study of the interrelationships of body composition, nutrition and physical activity is a longitudinal study of aging. It provides a description of these characteristics for participants in the Baltimore Longitudinal Study. It provides opportunity to relate changes in these basic characteristics of the individual participants to changes in other biochemical, physiological and psychological measurements. A variety of non-invasive techniques are employed. They include the Behnke Anthropometric Index, skinfold thickness measurements, height, weight, twenty-four hour creatinine excretion, total body potassium determination, basal metabolism determinations, Garn X-ray fat thickness measurements, a diet diary, and an activity questionnaire. Previously, measures of total body density and total body water have been made in longitudinal studies participants. Body density corrected for differences in body water content have been compared with the Behnke Index and other conventional anthropometric indices (such as ponderal index).

PHS-6040
(1-75)

Objectives: This project is designed to describe age differences and age changes in body composition, nutrition, and physical activity. Mechanisms of interaction of these functions and behaviors will be sought. The relationship of these measurements to other physiological, psychological and biochemical variables will be examined.

Methods Employed: Height, weight, and body circumferences of longitudinal study participants are obtained by standard anthropometric methods. Roentgenographic and anthropometric estimates of skeletal mass are combined with height, weight, and body circumferences to provide an estimate of body fat. Other estimates of fat include skinfold thickness measurements and fat thickness measurements from X-rays. Indices of lean body mass include: (1) basal metabolic rate determinations, (2) twenty-four hour urinary excretion of creatinine, (3) total body potassium, and (4) total body water and extracellular water determinations by indicator dilution. Nutrient intakes and activity calories are estimated from a diary and a self-administered questionnaire. All such measurements are repeated in the course of each subject's participation in the longitudinal program.

Major Findings: The Basal Metabolic Rate (BMR) of a healthy individual, measured at rest in a comfortable environment immediately after a night's sleep, is considered to represent the minimum energy transformation necessary to maintain body functions. Traditionally, the BMR value has been expressed in energy units per unit of body surface area and time based on the interpretation that energy in the form of heat is lost through the body surface. The BMR's of various animal species, including man, have been shown to be remarkably constant when expressed in those units. The assumption of heat loss failed to be supported, however, when data showed that animals of similar size and surface area, but living in two extremes of temperature environment, had the same rates of energy transformation.

The BMR test was for many years of clinical value in the diagnosis of metabolic disorders, chiefly those of thyroid malfunction. It has now been replaced by assays of thyroid hormone in the blood. While it was in use, BMR norms for men and women, based on many determinations on healthy individuals, served as standards for the evaluation of patients. Age was early recognized as an important factor since BMR decreases with age in adults at the approximate rate of 1 kcal/m^2/hr per decade of life. For lack of a better explanation, this decrease in energy requirement with age was interpreted as the gradual waning of activity of individual cells which culminates in death.

Recognition that a living body is made up of many different tissues, each with a different metabolic rate, led to alternate interpretations. One which enjoys popularity postulates that basal oxygen consumption decreases because the mass of active tissues in the body decreases with age. When basal oxygen consumption was related to mass of lean body tissues (body mass with fat mass subtracted) rather than to surface area, the age difference appeared to be cancelled. This finding confirms that fat tissue is

relatively inactive and suggested that the decline in mass of lean tissues was responsible for the decreased metabolic rate with age.

One of the largest single components of lean tissue is muscle. It produces creatinine, a compound derived from creatine which, as phophocreatine, stores energy for adenosine triphosphate synthesis. Once formed, creatinine diffuses out of the muscle and is excreted by the kidneys unchanged. To the extent that it is a product of muscle tissue alone and entirely excreted by the kidneys, the amount of creatinine output over a long period of time (24h) should be constant from day to day and bear relation to the muscle mass which produced it.

Basal oxygen consumption and 24 h creatinine excretion were compared in 966 participants in the Baltimore Longitudinal Study who had one or more visits for testing. The ratio of basal oxygen consumption to creatinine excretion increased with increasing age indicating that a smaller amount of muscle remained in older individuals relative to the total metabolizing cell mass. The relation of basal oxygen consumption to creatinine excretion was then calculated for three age groups (20-44, 45-64, 65-100). It resulted in equations for straight lines which were not significantly different in either slope or y-intercept. It was thus possible, by using one equation, to separate from the total basal oxygen consumption for each individual the basal oxygen consumption which is due to muscle alone.

While the average total basal oxygen consumption for the old men 90 years of age was 23% lower than that for the young in the 20's and 30's, the non-muscle portion was unchanged at about 124 ml/min for men of all ages studied. These results clearly indicate that the decrease in basal oxygen consumption with age is simply a result of the loss of muscle tissue.

It is of interest to quantify the amount of muscle represented by a given creatinine excretion. Lean body mass estimated from anthropometric measurements were available for 927 participants who also had 24-hour excretions determined. The relation between lean body mass and creatinine excretion was computed for the three age groups as previously done for basal oxygen consumption. The resulting lines had slopes which tended to decrease with increasing age. In units of kg per gram of creatinine excreted, the mean slopes for the three age groups 20-44, 45-64, and 65-100 were 20.8, 20.2, and 15.3, respectively. For the first two groups the values compared very well with literature values for infants, college students, and rats. The low slope for the oldest subjects suggests that old muscle produces, per unit mass, less creatinine than young muscle and that the amount of non-muscle tissue (bone and other organs) increase in weight with age, despite evidence to the contrary. Since anthropometric measurements reflect size rather than composition, replacement of active tissue with connective tissue in old muscle would go undetected by anthropometry but be reflected as reduced creatinine excretion. In view of these considerations, the conversion factors for the younger groups should be applicable to the oldest group. Muscle masses for longitudinal studies participants were 36.8 kg for young (20-39 yr), 33.5 kg for middle aged (40-59 yr) and 28.3 kg for old (60-79 yr).

Muscle has been identified as the tissue responsible for the decline in oxygen consumption with age. Muscle is lost in the longitudinal studies participants at the rate of 2.3 kilograms per decade.

The second round of data collection in the nutrition survey of longitudinal studies participants has been completed. The first round covered the period, March 1961 through September 1965, while the second round covered the period, March 1968 through June 1975. In both rounds of data collection, participants kept 7-day diaries of food eaten before or after their visits to GRC. Dietitians converted the diaries to food codes and amounts. Total calories per day, calories from protein, fat and carbohydrate, and daily amounts of 22 nutrients were computed with a conversion program based on U.S. Department of Agriculture food composition tables at the Computer Center of Washington University in St. Louis, Mo.

A preliminary examination of the data has revealed that concerns about possible differences in coding of the diaries or application of the conversion program between the first and second periods of data collection and analysis proved unfounded. Two hundred diaries collected during the first round were coded and converted during the latter half of the second round by four dietitians. Mean values for average daily caloric intake ranged from 2309 to 2410 calories per day as compared to an average of 2391 calories per day for 254 diaries coded and converted during the first round of data collection. None of the four dietitians provided values which differed significantly from the first round value.

There was a decline from the first round to the second round for calories and some nutrients. While total calories per day and calories from protein, fat and carbohydrate declined significantly from the first round to the second round, the proportion of calories from protein, fat and carbohydrate were similar for both rounds. Alcohol intake increased while cholesterol, calcium and vitamin A decreased from the first to the second round. The average intake of saturated and unsaturated fatty acids, oleic acid and other fatty acids decreased while the intakes of linoleic and linolenic acids made little or no contributions to the changes. Dietary intakes of the B vitamins were similar for the first and second rounds of data collection. It seems that the well-nourished participants in the longitudinal studies have as a group prudently decreased their food intake especially with respect to certain types of fat. The replacement of some of this reduction with a modest increase in alcohol intake should be examined further.

Significance to Bio-Medical Research and the Program of the Institute: Nutritional deficiencies in the aged are known to be common and are generally attributed more to the socio-economic deprivation of this group than to biological or physiological aging effects. The volunteers in the Longitudinal Study Group are not a deprived group--it may be characterized as upper-middle class and has a very high educational level. It, therefore, offers a unique opportunity to study nutritional status under very favorable conditions. The nutritional effects of biological

age per se may, therefore, be separated from what might be called "social aging."

Certain age changes in organ systems and various diseases are thought to be affected by diet, level of physical activity, and body composition. From the repeated assessment of these factors over time, it may be possible to determine their relative contributions to longevity and the maintenance of health and vigor in later life. Difficulties associated with obtaining retrospective estimates of eating habits, activity and body composition in the past make a prospective approach necessary for the collection of reliable information.

Proposed Course: Studies of diet, physical activity and body composition will continue. Data already collected will be further analyzed. Interactions of changes in body composition food intake, food composition, kind and amount of physical activity, disease, and age will be examined. Specifically, body fat and lean body mass estimates, nutrient intakes and physical activity category will be used in an analysis of risk of cardiovascular disease and of rate of aging of several organ systems.

Publications:

Rose, C. S., Gyorgy, P., Butler, M., Andres, R., Norris, A. H., Shock, N.W., Tobin, J., Brin, M., and Spiegel, H.: Age differences in vitamin B-6 status of 617 men. Am. J. of Clin. Nutrition. In Press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG. 00018-10 CPB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Marital, Sexual and Social Factors In Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. Martin	Sociologist	CPB NIA
OTHER:	S. M. Harman	Medical Officer	CPB NIA

COOPERATING UNITS (if any)

Baltimore City Hospitals

LAB/BRANCH
Clinical Physiology Branch

SECTION
Human Performance Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.25	.90	.35

SUMMARY OF WORK (200 words or less - underline keywords)

Systematic information concerning sexual, marital and social experience was obtained by structured interviews with males taking part in the GRC longitudinal study of aging. In the present analysis, limited to over 500 subjects aged 40-79 at report, correlations were computed between many of the variables derived from the interview in order to develop a statistical description of the structural features of the male sexual life history. Major findings include the demonstration of a substantial negative correlation between age at first coitus and number of coital partners before age 40, as well as a general lack of correlation between number of partners and measures of sex drive as had been assumed. The best predictor of sex drive proved to be respondent estimates of frequency of coitus in the first year or two of marriage. From the perspective of etiologic inquiry, future plans are to relate the various variables obtained from interviews to such diagnostic entities as coronary artery disease, hyperlipidemia and other diseases and disabilities.

PHS-6040
(1-75)

Objectives: Present goals are to: (1) discover whether coronary artery disease in longitudinal subjects, and whether other diseases or disabilities of unknown etiology, may be associated with any attribute of prior sexual, marital or social experience, (2) determine whether individual differences in level of sexual functioning may be related to other physiological, psychological or behavioral characteristics than those already considered, (3) interview subjects newly-admitted to the longitudinal program, and (4) reinterview subjects to update information on marriage and sexual functioning with special attention given to those participants who agree to take part in Dr. S. Mitchell Harman's study of gonadotrophin secretion, pituitary gonadotrophin reserve and Leydig cell reserve in relation to level of sexual function.

Methods Employed: The investigator has now completed initial interviews with nearly all subjects in the program regarding their history of marriage and sexual activity. In requesting these interviews, the investigator outlined study objectives in considerable detail, provided assurance of confidence and emphasized the voluntary nature of such a contribution. Over 650 subjects have now completed interviews; 14 declined to be interviewed.

To aid fluency of communication and to insure the accumulation of systematic information, questions were memorized by the investigator in addition to whatever codes were deemed necessary for the classification of responses. With a view to rapport and the generation of data not obtained elsewhere in the testing schedule, various aspects of residential, occupational, educational, religious, military and parental-home experience were reviewed before turning to questions concerning sexual conduct and marital adjustment. The data obtained are unique and are thought to be of excellent quality because of the high occupational and educational attainment of subjects and their evident interest in this aspect of the study.

Major Findings: To develop a statistical description of the structural characteristics of the male sexual life history, variables derived from interviews with subjects aged 40-79 were correlated with such key features of the life history as: age at initial marriage, age at first coitus, number of coital partners before age 40, and the sum of all coital and all sexual events reported for the 20-year interval between 20 and 40 years of age. The first two variables specify ages at onset of marriage and coitus; the third, number of partners, is an indicator of degree of exposure to infections that may be venereally transmitted; while the latter two variables constitute several operational measures of sex drive.

To gain partial control over the biasing effects of length of recall and generational differences, subjects were subdivided into age groups, 40-59 and 60-79, before computing coefficients of correlation. In general, the magnitude of the correlations obtained between key characteristics and their correlates have proved to be quite comparable. The following table illustrates the kinds of variables considered and their relationship with age at first coitus.

Correlates of Age at First Coitus

	Age at Interview		
	Total	40-59	60-79
No. changes M status bf 40	-.18(500)	-.19(293)	-.15(207)
Max. no. C events any week	-.23(500)	-.16(298)	-.26(202)
Freq. coitus, early marriage	-.27(500)	-.24(297)	-.28(203)
No. sexual events, ages 20-40	-.30(493)	-.24(294)	-.34(199)
Age first married	.38(505)	.39(298)	.34(207)
Sum, spouses' ages at marriage	.39(505)	.39(298)	.35(207)
No. coital events, ages 20-40	-.42(493)	-.41(294)	-.42(199)
Age first petting experience	.61(504)	.68(298)	.50(206)
No. coital partners before 40	-.68(505)	-.62(298)	-.71(207)

NOTE: M = marital; C = coital.

The most immediately useful, as well as unanticipated, finding was the strength of the negative correlation between age at first coitus and number of coital partners before age 40 (r = -.68). This relationship is of interest with respect to analyses designed to assess the hypothesis that a given disease may be due to some venereally-infectious agent. In this connection, age at initial coitus specifies the maximum length of incubation time whereas the probability of infection may be described by the variable, number of partners. Both variables will, therefore, need to be controlled in any analysis to test the suitability of such a hypothesis.

The variable, number of partners before 40 was also found to be largely independent of the quantity of both coital and total sexual activity reported for the interval between 20 and 40 years of age, contrary to the assumption of a substantial relationship in the literature. In the present analysis, the "best" single predictor of sex drive proved to be respondent estimates of the customary frequency of coitus in the first year or two of marriage. This latter estimate correlated .73 with the number of coital events reported to occur between 20 and 40 years of age, and correlated .70 with the quantity of all sexual events reported for the same period. Since both motivation and opportunity for sexual expression are maximal in early marriage, the pattern of coital frequency established at this time apparently influences level of activity for some years following marriage. Thus, coital frequency in early marriage can be employed as an indicator of sex drive in other contexts of inquiry.

Significance to Bio-Medical Research and the Program of the Institute: Various authors have assumed that early coitus, early marriage, unstable marriage, numerous coital partners and certain physiological characteristics are indicative of high sex drive. However, these assumptions receive little or only modest support in the present study. Empirical studies are clearly required for the interpretation of findings pertaining to

human sexuality. Moreover, the investigation of whether attributes of sexual or marital experience may have an etiologic role in chronic disease has been seriously neglected. The interdisciplinary nature of the longitudinal program at GRC provides an unusual opportunity to explore this type of question.

Proposed Course: In addition to conducting interviews and preparing the above materials for publication, plans are in progress to determine whether longitudinal subjects with a diagnosis of coronary artery disease differ from their controls with regard to past sexual, marital and social experience. The analysis is expected to be the first of a similar series of studies involving: hypertension, hyperlipidemia, diabetes, stroke, arthritis, prostatic hypertrophy and erectile failure.

Publications:

Martin, C.E.: Marital and Sexual Factors in Relation to Age, Disease and Longevity. In Wirt, R.D., Winokur, G., and Roff, M. (Eds.): Life History Research in Psychopathology, Volume 4, Minneapolis, Minn., University of Minnesota Press, 1975, pp. 326-347.

Martin, C.E.: Frequency of Male Sexual Activity in Relation to Measures of Physiological Function and Body Composition. In: Proceedings of the 10th International Congress of Gerontology, Volume 1, Plenary Sessions, Symposia. The Congress, Jerusalem, Israel, June 1975, pp. 359-361.

Martin, C.E.: Sexual Activity in the Ageing Male. In Money, J. and Musaph, H. (Eds.): Handbook on Sexology, Amsterdam, Excerpta Medica Foundation (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00021-13 CPB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Dermatoglyphics in: 1. Populations 4. Families
2. Medicine 5. Twins
3. Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	J.T. Schwartz[2]	Special Asst. to the Director	
	R. Garruto	Senior Staff Associate	CNS NINCDS
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	R.W. Hornabrook[4]	Director	
	C. Bartsocas[5]	Associate Professor of Pediatrics	
	A. D'Alessandro[6]	Director	
	M.T. Newman[7]	Professor of Anthropology	
	W.W. Greulich[8]	Professor of Anatomy	
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(continued on following page)

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SECTION
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INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.85	0.50	0.35

SUMMARY OF WORK (200 words or less - underline keywords)

This project represents a joint collaborative effort, involving the WHO and other national and international biological laboratories to coordinate the evaluation and interpretation of the available dermatoglyphic data. Specifically the objectives of this project are: 1) to study the distribution of dermatoglyphics among the various human populations (population dermatoglyphics); 2) to establish the dermatoglyphic frequencies in normal control samples (control dermatoglyphics); 3) to establish dermatoglyphic markers in various diseases (clinical dermatoglyphics); 4) to study the dermatoglyphics of the aged; 5) to study the genetics of dermatoglyphics; and, 6) to utilize dermatoglyphics as an added tool in twin diagnosis (twin dermatoglyphics).

PHS-6040
(1-75)

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University of Washington
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Project Description:

Objectives: This project represents an extensive collaborative effort, in conjunction with WHO and other National and International institutions, to study all aspects of dermatoglyphics. The specific objectives of this study are: (1) to establish the distribution of dermatoglyphic features in various populations, with special emphasis on primitive and other isolated groups in the South Pacific and other parts of the world. (2) To establish associations between dermatoglyphic features and specific clinical anomalies. (3) To study the dermatoglyphic frequencies in different age groups. (4) To investigate the genetic aspects of dermatoglyphics through family and twin data.

The overall purpose of these studies is to utilize the dermatoglyphic markers in an effort to study the genetic structure of different populations, and to provide additional tools for studying the etiology of certain diseases, especially those influenced by intrauterine disturbances during the first half of pregnancy.

Methods: Digital and palmar prints collected by different groups through various methods are sent to our laboratory for evaluation and interpretation of the results.

Major Findings: The main findings of this collaborative study: (1) Established panels of the only dermatoglyphic frequencies for normal Caucasians and Negro Americans which can be used as controls in clinical dermatoglyphic studies. (2) Provided associations between dermatoglyphics and Down's syndrome, leukemia and other diseases. (3) Provided additional evidence for determination of twin zygocity. (4) Showed through twin studies that intrauterine disturbances during the first four months of pregnancy have considerable effect upon the final formation of dermatoglyphics and their post natal frequencies. (5) Showed that, with the possible exception of the modal types of the D line and the aberrant simian creases, age does not affect the dermatoglyphic frequencies. (6) Established the dermatoglyphic frequencies in several isolates not previously available.

Significance to Bio-Medical Research and the Program of the Institute: The establishment of normal control dermatoglyphic frequencies for both the Negro and Caucasians will provide a uniform standard for evaluating the dermatoglyphic changes in different diseases. Dermatoglyphic associations provide additional markers in the overall effort to establish the etiology of certain diseases. This is especially true since there is only a short and specific time in life, the first four months of development, during which the dermatoglyphics are vulnerable to any type of change. Finally, the dermatoglyphic frequencies provide additional genetic markers in the overall genetical and medical evaluations of primitive populations.

Proposed Course: To continue this project by further evaluating the data at hand and by collection of additional clinical and population dermatoglyphic data.

Publications:

Wertelecki, W. and Plato, C. C.: Palmar Flexion Creases in Birth Defects and Leukemia: A Survey of 5000 Individuals Stressing Normal Ethnic, Sex and Familial Differences. Proceedings of the XIV International Congress of Pediatrics (Pediatria XIV): 10:111-113, 1974.

Wertelecki, W. and Plato, C. C.: Subclassification of Normal Palmar Flexion Creases.--A Tool to Assess Prenatal Development. Proceedings of the XIV International Congress of Pediatrics (Pediatria XIV) 10:108-110, 1974.

Greulich, W.W., Berg, Jr., W.R., Culotta, C.S., Plato, C.C., and Yannet, H.: A case of Mongolism in DZ female twins studied at 10 and then at 43 years of age. Acta Gen. Med. et Gem. (Roma) 24:47-61, 1975.

Ahuja, Y.R., Murty, J.S., Plato, C.C. and Schwartz, J.T.: Inheritance Studies of c-t and a-t Intertriradial Distances on the Palm by Means of Twin Pair Analysis. Proceedings of the Second Annual Conference of the Indian Society of Human Genetics. (Calcutta, India), November 1975.

Plato, C. C., Brown, H., Gajdusek, G.: The dermatoglyphics of the Elema people from the Gulf District of New Guines. Amer. J. Phys. Anthrop., 42:241-250, 1975.

Plato, C. C., and MacLennan, R.: The dermatoglyphics of the Maprick Sub-District of the Sepik District of New Guinea. Zeits. Morph. Anthrop. (Stuttgart): 66:208-216, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00022-01 CPB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Epidemiological Investigations of Osteoarthritis of the Hand

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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SECTION

Human Performance Section

INSTITUTE AND LOCATION

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.85	0.50	0.35

SUMMARY OF WORK (200 words or less - underline keywords)

The overall objective of this project has been the epidemiological study of osteoarthritis or degenerative joint disease of the hand. Specifically, this project deals with: 1) the joint-digit prevalence of the disease; 2) its familial aspects; 3) its bilateral symmetry; 4) its association with selected physiological and anthropometric variables; and, 5) longitudinal changes in prevalence of the disease.

PHS-6040
(1-76)

Objectives: Presently, osteoarthritis of each hand is graded by the grade of its most severely affected joint. The objective of this investigation has been to study the epidemiological aspects of osteoarthritis of the hand by grading and evaluating each type of joint separately. This type of analysis will offer a more precise epidemiologic description of the disease and will provide further information for determining its etiology.

The present investigation involves four separate studies: (1) Joint-Digit prevalence. To establish age specific prevalence rates for osteoarthritis for each joint of each digit, in an effort to obtain evidence for preferential involvement. (2) Familial Aspects. (3) Bilateral symmetry. (4) Associations of the disease with several selected anthropometric and physiological variables. (5) Longitudinal Aspects.

In addition to the above a preliminary study was made to ascertain the prevalence of osteoarthritis of the thoracic spine.

Methods Employed: Radiographs were graded separately for each of the proximal and distal interphalangeal joints utilizing the internationally accepted grading system of J. H. Kellgren. Grades 0, 1 were considered normal and grades 2, 3 or 4 were considered as having osteoarthritis (affected). With the exception of bilateral symmetry, all other studies involved X-rays obtained from the subjects of the longitudinal study, of the Gerontology Research Center. The GRC data involved only left hand X-rays. For the bilateral symmetry study, we graded the X-ray films of both hands from one hundred male and one hundred female participants of the Tecumseh Study.

Major Findings: Prevalence of osteoarthritis of the hands of grades 2, 3 and 4 varied with age. In subjects under 40 years of age up to 2.6% had affected joints, in subjects between 40 and 59 years old as much as 24.7% had osteoarthritis, while in subjects over 60 years of age nearly 60% had an osteoarthritic joint. These highest prevalences found in the distal joint of the fifth digit are in agreement with other surveys in the literature. Differences in prevalence rates between digits and joints are shown in the following table of % positive (grades 2,3,4):

Joint-Digit	Age Groups		
	less than 40	40-59	60 & older
Distal			
D1	1.8	6.6	29.1
D2	2.2	17.8	49.8
D3	1.8	11.7	38.5
D4	0.9	10.6	33.8
D5	2.6	24.7	58.5
Proximal			
M1	0	1.9	8.7
P2	0	2.4	14.7
P3	0	5.3	15.1
P4	1.8	2.1	17.4
P5	1.3	6.6	31.8

There is a well defined preferential involvement for osteoarthritis of the hand. (a) As a group, the distal interphalangeal joints of the hand are more frequently and more severely affected than the proximal. (b) Within each joint group there is a specific interdigital preferential involvement in terms of prevalence as well as severity. (c) The order of interdigital preferential involvement is not the same in the two groups of joints (i.e., proximal or distal interphalangeal).

Among the additional studies performed, the sib comparison gave some evidence for familial tendency for osteoarthritis. However, the size and the age distribution of the "sib" sample precluded convincing findings.

The bilateral studies indicated consistently a higher prevalence and more severe form of osteoarthritis in the right hands. The exact contribution of handedness to this finding could not be ascertained from the present data.

The comparison of the osteoarthritic status to variables suspected to be or shown in other studies to be associated with the disease also produced interesting results. It was shown for the first time that activity-related energy expenditure, wrist diameter, forearm circumference, and ponderal index are related to the presence of the disease in the proximal and metacarpophalangeal joints only, while the most prevalent type, osteoarthritis of the distal interphalangeal joints, is not related to any of these variables. This suggests that there is a different etiology of development of osteoarthritis in the distal as opposed to the more proximal joints.

Longitudinal results were obtained from 475 participants who had two or more hand x-rays. Subjects were separated into age groups based on age at first visit. Follow-up comparisons were made as little as two years and as much as 16 years following the initial x-rays. In general, changes followed the prevalence patterns. Subjects of all ages moved to higher osteoarthritic grade levels with increase in age. The overall results indicate that osteoarthritis of the hand is a slowly progressing disease. The prevalence-severity index, developed for this study, indicates that the highest rate of change is encountered at the age of 60 years and older in the distal interphalangeal joints where there is, on the average, a 1.25 grade increase at the longest follow-up intervals (12-16 years). The results of this study give for the first time an assessment of the rate of progress of osteoarthritis of the hand.

A preliminary evaluation of chest x-rays of 280 participants radiographed at GRC during the past 8 months used the Kellgren criteria of classification. Seventy-five individuals or 25.7% had osteoarthritis of the thoracic spine. Of these 75, fifty-seven individuals or 83% also had osteoarthritis of the hand.

Significance to Bio-Medical Research and the Program of the Institute:
The unique approach to the study of osteoarthritis and the ensuing

results will provide additional tools for future studies dealing with the etiology of osteoarthritis.

Proposed Course: To prepare manuscripts for publication. Follow-up with a detailed study of osteoarthritis of the spine and its relationship with the presence of the disease in the hands and other joints.

Publications: None

NIA Annual Report
July 1, 1975 through June 30, 1976
Gerontology Research Center
Laboratory of Cellular and Comparative Physiology

The long term goals are: (a) to conduct studies on the cellular and molecular etiology of cellular aging of the immune and related systems, (b) to develop methods for early detection of signs of cellular aging and (c) to develop methods to control the harmful changes associated with aging which can lead to eventual cellular paralysis or death.

Our current research activities have been centered on the cellular and molecular etiology of aging. Research activities have also been extended to the areas on pathogenesis of aging, the development of methods for early detection of signs of cellular aging and the development of methods to control cellular senescence. Immunocompetent cells and fibroblasts served as primary and secondary models, respectively.

During the middle of the fiscal year (1/76), a catastrophic, parainfluenza (Sendai virus), epizootic infection occurred in our mouse facility (5,000 aging mice and 4,000 experimental mice). Needless to say, the widespread infection, which originated from a shipment of presumably healthy normal mice purchased from VRB, NIH, seriously affected our research activities, including 7 individual projects which had been in existence for 2-4 years, and 3 projects which were initiated in FY 1976. Most of these projects by necessity will be held in abeyance until an adequate supply of uninfected, genetically defined, aged mice can be made available to the laboratory again. In any event, our accomplishment prior to the infection is as follows.

A. Cellular and molecular etiology of aging

One of the hallmarks of aging is the increase in variability of physiological parameters between individuals, including those of the immune system, which would suggest that several factors are responsible for the increase in variability. Thus, it was not surprising when we demonstrated earlier that the decline in immune capacity of aging mice results from deficiencies in both the immune cells and their milieu. Current studies indicate that the cellular changes responsible for immunosenescence include: (a) a loss of certain immune cells, (b) an increase in suppressor cells, and (c) an alteration in functional efficiency of immune cells. Concerning the latter, it could be reflective of a genetic alteration. Supportive of this view is the demonstration that chromosomal alteration increases with age among cells of the lymphohematopoietic system.

Studies of stem cells revealed that not only does the clonal growth capacity decline with age, but that old stem cells remain characteristically old even after they are allowed to self-replicate in the bone

marrow of young mice for an extended period of time. On the other hand, young stem cells can be aged precociously by allowing them to self-replicate in old mice. These results indicate that the difference in clonal growth capacity of stem cells is reflective of differences in the maturation stages of the differentiation vector which is difficult to reverse, as with other morphogenetic expression of differentiation.

Studies on the regulation of T cell-independent humoral immune response indicate that while old mice can respond as vigorously as young mice, their spleen cells do not. The latter seems to be correlated with the level of antigen-nonspecific glass-adhering suppressor cells.

Studies on membrane receptors indicate that (a) peripheral blood lymphocytes of old humans are more fragile than those of the young when exposed to solutions of varying osmolarities, (b) lymphocytes from the thymus, spleen, and lymph nodes of old mice tend to be more fragile than those of young mice during routine manipulation for tissue culture, and (c) lymphocytes become extremely labile following exposure to Sendai virus infection with cells from old mice being more severely affected than cells from young mice.

Continuing studies on the mechanism of removal of senescent cells by macrophages indicate that the physiologic IgG autoantibody is polyclonal and that those eluted from in situ aged red blood cells can initiate phagocytosis of neuraminidase-treated young red blood cells.

Studies on aging of human fibroblasts revealed that (a) skin fibroblasts from old donors have a diminished self-replicative capacity relative to those from young donors, (b) senescent fibroblasts can be separated from adult fibroblasts by velocity sedimentation and (c) the elevated RNA content of senescent fibroblasts is not due to an increase in its synthesis. Studies on maternal age effects in mice revealed that (a) frequency of aneuploid embryos increases with increase in maternal age in 5 inbred strains of mice and (b) a correlation exists between chromosomal and morphological abnormality.

Emphasis on future studies will not only be more mechanistically oriented but will focus on both extrinsic and intrinsic factors which influence the differentiation pathways of immunocompetent cells.

B. Immunopathogenesis of aging

Five types of studies were performed: (a) characterization of the immune system of autoimmune-prone aging NZB mice, (b) precursors of old-age associated leukemic cells in humans, (c) fidelity of the immune system of immunodepressed, x-irradiated, allogeneic bone marrow chimeras, (d) mechanism of regulation of expression of autoimmune disease, (e) the role of T cells in myeloma induction, and (f) consequence of immunorestoration of old mice on their life span and pathology. Major findings

of study (a) revealed that (i) the development of cytolytic lymphocytes decreases with age in both autoimmune-prone and resistant strains of mice, (ii) the decline is inversely related to the development of T suppressor cells, (iii) the suppressor cells act by releasing a humoral factor which is H-2 nonspecific, and (iv) the suppressor cells can be induced-by H-2 difference alone. The major findings of study (b) indicate that while T cell deficiency is induced immediately after induction of the chimeric state, B cell deficiency is not manifested until later in life. The major findings of study (c) indicate that "hairy" leukemic cells are derived from either B or plasma cells. No meaningful results were obtained from the latter 3 studies prior to the infection, as they were initiated in FY 1976. We are hopeful that these studies can be reinitiated with a minimum of lag time as they are crucial to our understanding of immunopathogenesis of the aged.

C. Methods for early detection of cellular aging

Three types of studies were performed: (a) in vitro immune functional indices which correlate best with those in situ, (b) correlation of immunologic indices of the peripheral blood and those of the spleen and lymph nodes and (c) development of the rat as the animal model to study longitudinal immunosenescence.

Studies on in vitro correlates of in situ immunologic activities of aging mice were revealing. Thus, it was noted that quantifiable kinetic data are difficult to obtain with use of many of the standard short-term culture methods. This could be due to the fact that initiation of immune responses generally requires the participation of two or more types of immune cells. In addition, the culture conditions may have been selectively favoring one of the immune cells. Further effort must be expanded in defining the number and proportion of immune cells involved in any given in vitro immune response. Another finding of interest is the demonstration that stem cells and B cells of adult mice may be sharing a common immunogenic surface receptor. This would suggest that certain biologic activities of stem cells and B cells can be differentiated with judicious use of the reagent.

Studies on the correlation between immunologic activities of the peripheral blood and those of the spleen and lymph nodes have been limited to the humans. These studies revealed several interesting findings, including the observation that the plant mitogenic index of these tissues are comparable and that the immunologic efficiency of the blood can be increased by removal of polymorphonuclear leukocytes and red blood cells. Interestingly, it was also noted that certain immunologic parameters of human and mouse lymph nodes and spleens are comparable (e.g., T cell frequency and responsiveness to plant mitogen).

The rat was chosen as the animal model for many compelling reasons (i.e., it ages rapidly, it is relatively inexpensive to maintain, it has sufficient body mass to allow serial sampling of blood, our immunopathogenic and endocrinologic knowledge is vast, etc.). The results of

current studies indicate that, while the rat lymphoid tissue, unlike that of the mouse and the human, is more difficult to culture *in vitro*, it nevertheless can be shown to manifest *in vitro* functional immunosenescence.

Based on our recent progress in this area, we are confident that we will be able to develop simple, sensitive, quantifiable, miniaturized assays of immune cells which are reflective of the immune status of individuals *in situ*, and therefore are essential to our understanding of the regulation of immunosenescence in humans.

D. Methods to control cellular senescence.

Three broad approaches have been undertaken since the inception of this program two years ago: chemical therapy, cellular therapy, and dietary manipulation.

In our chemical therapy studies, we have continued to assess the immunorestorative effects of 2-mercaptoethanol, a reducing agent. Previously, we demonstrated its immunorestorative effects in *in vitro* cultures. Our current studies indicate that the beneficial effects can also be demonstrable in intact old mice. Studies on its effect on the life span and pathology of aging mice were discontinued because of the Sendai epizootic infection.

In our cellular therapy studies, we have continued to assess the long-term immunological effects of the combined newborn thymus graft-young adult bone marrow stem cell injection therapy of old mice. In addition, studies on its effect on the life span and pathology were also initiated. Unfortunately, these studies, too, had to be discontinued.

The data of our recently completed project on dietary manipulation, which focuses on the effects of protein restriction, are now being analyzed. We are hopeful that the results will contribute to our understanding of the effects of dietary manipulation of immune activities, life span, and pathogenesis of aging.

Recently, we recruited a molecular biologist to develop and conduct research on the molecular etiology of aging of the neuroendocrine-thymus axis system, with focus initially on effects of age on the fidelity of neuronal and thymic DNA. Undoubtedly, the addition will have a synergistic effect on the overall performance of the laboratory because: (a) the thymus appears to be the pacemaker of immunosenescence, (b) a two-way regulatory communication axis exists between the neuroendocrine system and the immune system, and (c) an effective interaction between cellularly and molecularly oriented personnel is anticipated.

The Laboratory of Cellular and Comparative Physiology has just completed its fourth year under a new set of goals. Therefore it should have attained its "steady-state" level of creative productivity. Unfortunately, the recent Sendai epizootic infection has disrupted this timetable. The success of regaining the momentum in scientific activity as it existed prior to the incident, of course, will depend upon how soon the Institute can develop an animal facility with an adequate supply of uninfected, genetically defined, aging mice.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-AG-00081-04-LCP

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Age Effects on Proliferation and Differentiation of Immune Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. Makinodan	Chief, LCCP	LCP NIA
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	M. Heidrick	Assistant Professor	Univ. of Nebr.
	K. Hirokawa	Associate Professor	Tokyo Med. & Dent. Univ.

COOPERATING UNITS (if any)

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LAB/BRANCH

Laboratory of Cellular and Comparative Physiology

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INSTITUTE AND LOCATION NIA, NIH, Gerontology Research Center, Baltimore City Hospitals, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.4	1.05	1.35

SUMMARY OF WORK (200 words or less - underline keywords)

The long term goal of this project is to understand the cellular and molecular mechanisms for loss of immunologic vigor with age and to promote immuno-restoration. Present studies are focused on: (a) cellular etiology of immunosenescence, (b) the development of simple sensitive methods to assess the immunologic potential of peripheral blood cells and (c) the development of immunorestorative methods. Unfortunately much of the work has been curtailed as a result of a Sendai parainfluenza outbreak in our aging animal facility.

Objectives: The proliferative and differentiation capacities of immunocompetent cells decrease with increasing age. Consequently certain normal immunologic functions decline. Functional alteration of precursor cells may reflect a genetically programmed postmaturation event or a stochastic event. It may also reflect a change in the regulatory factors. The objectives of this project are (a) to determine the mechanisms responsible for functional alterations of immunocompetent precursor cells, (b) to develop simple and sensitive methods to assess the immunologic potential of peripheral blood, and (c) to develop methods for controlling age-related decline in normal immunologic functions.

Methods Employed: The proliferative capacities of bone marrow stem cells of young and old mice were assessed *in vivo* by determining their colony forming and proliferative activities in young and old recipients. The growth capacity of bone marrow stem cells from young and old long-lived mice was assessed in the spleen of x-irradiated young and old syngeneic recipients by determining: (a) the number of stem cells colonizing the spleen, (b) the rate of incorporation of ^{125}IUdR by proliferating colony cells, and (c) the number of cells present in the largest colonies at the end of the growth phase. To assess the nature of cellular changes responsible for the decrease in humoral immune activity in old mice, their spleens were assessed individually in the presence of indicator young cells. Attempts to determine the immunorestorative effects on the life expectancy of old mice were made by grafting newborn thymic tissue, by either injecting young stem cells, by the combined newborn thymic graft-young stem cell injection treatment, or by injecting low doses of mercaptoethanol into long-lived old mice. The immunologic potential of peripheral blood will be assessed for its mixed lymphocyte cultures, cytotoxic lymphocytes, migration inhibtion, mitogenicity, and T-cell-dependent immune response indices.

Major Findings: Due to the Sendai parainfluenza epizootic event which occurred 1/76, most studies were ruined and, of these, only a few can be repeated for logistical reasons. The most severely affected studies concerned the long term effects of immunorestoration. Fortunately, a few studies were completed before the outbreak, and their major findings are as follows.

The clonal growth capacity of stem cells declines with age. Moreover, the spleen-seeking and spleen colony growth capacities of old stem cells remained characteristically old even after they were allowed to self-replicate in the bone marrow of young recipients for an extended period of time. On the other hand, the spleen colony growth capacity of young stem cells could be reduced by allowing them to self-replicate in old recipients.

Several types of cellular changes may be responsible for the decrease in humoral immune activity in old mice. In about 65% of the old mice the reduced response appears to be due to emergence of suppressor cells, about 30% to reduction in number of at least one type of immune cell that exists in excess in young mice and about 10% to reduction in number of immune cells and/or to a decrease in their functional efficiency.

Significance to Biomedical Research and the Program of the Institute: The decline of functional immune activities with age has an obvious effect upon senescence in general and extension of health. If the cell types and regulator factors which are responsible for the decline can be identified and characterized, this will be a significant step toward determining the cause for the decline and approaches to control the decline. Moreover, a comprehensive understanding of the effects of aging on the immune system will contribute to other vital tissue and organ systems, especially those involving cells undergoing proliferation and differentiation.

Proposed Course: Efforts will continue to focus on the mechanisms responsible for functional alterations of immunocompetent precursor cells and on the development of methods for controlling the decline in normal immune functions. Initially, the animal facility must be re-established with aging mice, and this will take at least two years.

Publications:

Albright, J.F., Dietchman, J.W., Hassell, S.A. and Ozato, K.: Differential antibody production by adherent and nonadherent spleen cells transferred to irradiated and cyclophosphamide-treated recipient mice. J. Reticuloendothel. Soc. 17: 195-209, 1975.

Hirokawa, K.: Characterization of age-associated kidney disease in Wistar rats. Mech. Ageing Develop. 4: 301-316, 1975.

Hirokawa, K., Albright, J.W. and Makinodan, T.: Restoration of impaired immune functions in aging animals. I. Effect of syngeneic thymus and bone marrow grafts. Clin. Immunol. Immunopath., in press.

Hirokawa, K. and Makinodan, T.: Thymic influence on T cell differentiation. J. Immunol. 114: 1659-1664, 1975.

Kay, M.M.B. and Makinodan, T.: Immunobiology of aging: Evaluation of current status. Clin. Immunol. Immunopath., in press.

Makinodan, T.: Biology of Aging: Retrospect and Prospect. In Makinodan, T. and Yunis, E. (Eds.): Immunology and Aging. New York, Plenum Press, in press.

Makinodan, T.: Immunity and Aging. In Birren, J.E. (Ed.): Handbook of the Biology of Aging. New York, Van Nostrand Reinhold Co., in press.

Makinodan, T.: Immunobiology of aging. J. Amer. Geriat. Soc., in press.

Makinodan, T., Albright, J.W., Good, P.I., Peter, C.P., and Heidrick, M.L.: Reduced humoral immune activity in long-lived old mice: an approach to elucidating its mechanism. Immunology, in press.

Editor: Makinodan, T. and Yunis, E. (Eds.): Immunology and Aging. New York, Plenum Press, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-AG-00085-04-LCP

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Diet Probes to Study Aging Immunologic and Biochemical Functions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. H. Stoltzner	Staff Fellow	LCP NIA
OTHER:	T. Makinodan	Chief, LCCP	LCP NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Cellular and Comparative Physiology

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.05	0.85	1.2

SUMMARY OF WORK (200 words or less - underline keywords)

Protein restriction, in various degrees and duration, has resulted in increased life expectancy in older mice. This finding is being correlated with a number of assays in an attempt to understand the mechanism of this life prolongation effect.

PHS-6040
(1-75)

Project Description: Z01-AG-00085-04-LCP

Objectives: Over thirty years ago, it was discovered that moderately severe caloric restriction in developing rats results in significantly prolonged lifespan, up to 50% greater than normally fed control animals. It is the purpose of this study to measure the effects of five dietary regimens maintained in mice from the time of weaning as they age. In addition to analyses of longevity and body weight, the investigation will correlate a variety of immunologic and biochemical indices among the dietary groups.

Methods Employed: The five dietary groups utilized in this study are: (1) 24% protein from weaning until death or sacrifice; (2) 24% protein from weaning until 128 days, then switch to 8% protein diet until death of sacrifice; (3) 24% protein from weaning until 128 days, then switch to 4% protein diet; (4) 8% protein diet from weaning (3 weeks); and (5) 4% protein diet from weaning.

Fifty (50) mice from each group of animals placed on the five dietary regimens have been set aside for longevity analysis. The remaining mice are being utilized for a variety of biochemical and immunologic assays that are standard in this laboratory and are now in progress. These studies include: (1) weekly body weights; (2) daily assessment of mortality; (3) monthly dermatitis index; (4) sacrificial biochemical studies of (a) protein determinations on liver cytosols and kidney homogenates, (b) kidney catalase determination, and (c) liver aldolase determinations; (5) sacrificial immunologic studies of (a) splenic lymphocyte proliferative responses to three mitogens and allogeneic tissue, and (b) primary SRBC antibody and plaque response; (6) other studies of (a) organ weights, (b) selected histologic studies, and (c) hematocrit, peripheral white blood count, serum protein.

The sacrifice schedule was: 12 months, 3/19-4/6/75; 18 months, 4/14-4/30/75; 4 months, 4/20-5/5/75; and 24 months, 9/1-9/20/75.

Major Findings: The carefully executed weekly body weight determinations have clearly demonstrated differences between the control animals, the two switch groups and the straight 8% and 4% mice. The weight range is 34 grams for the controls and 25 grams for the 4% group, and these differences have been maintained even into old age at 24 months. There is also a difference in mortality between the control and the protein restricted groups, particularly those animals on the 4% protein diet. The relative significance of these differences will be shortly ascertained by the detailed longevity study utilizing all 1000 mice in a computer program presently being written. All but the final "zero point" have been completed. These data, in view of their quantity, await a computer approach to aid the analysis. Preliminary examination of the data suggests a markedly diminished primary IgG antibody response in the most protein restricted mice, in spite of the fact that their median age is greatest. The sucrose dependent liver aldolase induction by high sucrose, which was purposefully varied inversely with protein in the diet, is diminished in old age, in the restricted groups when compared to the control. All

groups have passed the 50% mortality point, and less than 20% of the control animals, the group with the greatest number of deaths, are alive.

Significance to Biomedical Research and the Program of the Institute: Dietary manipulation is a very important if not the only method for prolonging normal life expectancy in certain mammals. This study, utilizing a rather comprehensive experimental design, shall define the biochemical and immunologic effects of protein restriction in mice, and by the nature of these studies provide insight into mechanisms of these effects. Additionally, it will correlate the experimental findings with morphologic and longevity analyses.

Proposed Course: The diet project is now in its concluding stages Ninety per cent of the assays are completed and tabulated and only about 200 animals of the initial 1,000 finally used for the project are alive. It is expected that all these mice will die before July 1977, and if the January 1975 cohort of mice is not included in the final mortality analysis, then all the remaining animals should expire by August 1976.

Presently, the major scientific tasks confronting the project are the tabulation, computation and analysis of an immense quantity of data, estimated to be in excess of 10^6 digits. Utilizing GRC computational hardware and the helpful assistance of several consultants in the computer, statistical and biochemical fields, it is hoped that this task can be well on its way to completion by early May. Portions of the anticipated publications are now being written.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-AG-00086-02-LCP

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

The Rat as a Model for the Immunologic Study of Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. H. Stoltzner	Staff Fellow	LCP NIA
OTHER:	T. Makinodan	Chief, LCCP	LCP NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Cellular and Comparative Physiology

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.25	0.25

SUMMARY OF WORK (200 words or less - underline keywords)

Utilizing the rat as a model of mammalian aging, quantification of age-dependent decline in lymphocyte proliferative ability when stimulated by lectins and allogeneic cells is being performed.

PHS-6040
(1-75)

Project Description:

Objectives: The rat is a typical mammal that is senescent by three years of age. It is the purpose of this project to carefully document declining function in the compartments of the immunologic system as measured by several standard indices. Of particular interest is comparing the function of blood lymphocytes with other aspects of the immunologic system, since blood is a tissue that can be easily and repeatedly obtained over time from the same subject animal.

Methods Employed: Utilizing various aged (weanling to > 24 month) male Fisher 344 rats, spleens, thymuses, and blood lymphocytes are harvested and cultured for three days in the presence of phytohemagglutinin, pokeweed, conconavalin A or xenogeneic irradiated lymphocytes. Proliferative responses as measured by incorporation of ^{3}H-thymidine are obtained by standard procedures in the laboratory.

Major Findings: This work has progressed, although at times interrupted by other more pressing laboratory obligations, and should be completed at the end of the fiscal year. Using the Fisher rat, this experiment has noticed considerable variability in the culture results from animal to animal, a finding that has been seen by another investigator in our laboratory using the rat model. A great deal of attention has been given to a variety of culture conditions, and presently a satisfactory culture system has been developed. In spite of rather wide individual variability within the age cohorts, and restricted numbers of individuals in each group, there seems to be a profound decline in lymphocyte proliferative activity as measured by thymidine uptake of aged rats when compared to young adults.

Significance to Biomedical Research and the Program of the Institute: Most mammalian organ systems, including the immunologic one, have declining functional capabilities with age. Changes in the immunologic system are particularly important since diminished immunologic activity can be directly correlated with increased susceptibility to a variety of infectious agents as well as the dramatically increased incidence of neoplasia with age, both of which are principal causes of death.

The purpose of this project is to define the rat as a suitable model system for the study of immunologic aging. The rat is an ideal animal since it ages rapidly, is relatively inexpensive to keep, but has sufficient body mass to permit serial sampling of blood and other tissues over time.

Proposed Course: Presently, various studies cited above are being concluded, with the proposed completion date before the end of this fiscal year. There remains the probability of combining these results with other investigators who have obtained data with the Wistar rat.

Publications:

Stoltzner, G. and Makinodan, T.: Age Dependent Decline in Proliferation of Lymphocytes. In Cristofalo, V.J., Roberts, J. and Adelman, R.C. (Eds): Explorations in Aging. New York, Plenum Press, 1975, pp. 21-37.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-AG-00087-03-LCP

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Mechanism of the Parental Age Effects

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: E. L. Schneider Medical Officer, PHS LCP NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Cellular and Comparative Physiology

SECTION

INSTITUTE AND LOCATION NIA, NIH, Gerontology Research Center, Baltimore City Hospitals, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

SUMMARY OF WORK (200 words or less - underline keywords)

With increasing maternal age, there is an exponential increase in the frequency of children born with chromosomal disorders. A mouse model has been utilized to examine the mechanisms of this maternal age effect. The frequency of chromosomally abnormal embryos obtained from retired breeders (8+ months old) was significantly greater than that obtained from young (3-5 month old) females [32/244 (13%) vs 5/229 (2%)]. The genetic component of this maternal age effect was examined by measuring the frequencies of chromosomally abnormal fetuses derived from retired breeders of several inbred mouse strains (A/J, C3H, C57Bl/6, CBA). In each of these inbred strains, a significant maternal age-related increase in chromosomally abnormal embryos was observed, indicating that simple genetic factors may not play an important role in the maternal age effect. The immunological component of the maternal age effect was analyzed by inducing immune incompetence in A/J mice by thymectomy and then determining the frequency of chromosomally abnormal embryos in old thymectomized females. The results revealed no difference in the frequencies of chromosomally abnormal embryos derived from thymectomized animals when compared to age-matched controls (17.1% vs. 16.7%). Studies are currently in progress to examine other proposed etiologic agents for the maternal age effect as well as to examine chromosomally aneuploidy as a function of aging in paternal gametes (sperm).

PHS-6040
(1-75)

Project Description:

Objectives: It has been clearly established that with increased maternal age there is a greatly increased risk of children being born with chromosomal disorders. Despite considerable speculation about the cause of this maternal age effect, research to delineate the mechanisms of this effect has been limited by the practical as well as ethical considerations of human experimentation. This problem has been approached in this laboratory by utilizing the mouse as an animal model since it has been demonstrated that with increased mouse maternal age there is an increased frequency of chromosomally abnormal fetuses.

Studies were initially directed at examining the genetic and immunologic components of the maternal age effect. The presence of genetic determinants of the maternal age effect was assessed by analyzing the frequencies of chromosomally abnormal fetuses from old and young mice from several genetically well-characterized inbred strains.. The immunologic component was examined by analyzing the frequency of chromosomally abnormal offspring in two mouse inbred strains which develop altered immune competence (NZB, A/J). In addition, immune competence was induced in one of these strains (A/J) by thymectomy and the effect of this manipulation assessed on the maternal age effect.

Methods Employed:
1. Young females and retired female breeders from five mouse inbred strains (A/J, C57Bl/6, C3H, CBA/CaJ, NZB) were mated with young males. Pregnant females were sacrificed between ten and thirteen gestational days. Chromosome preparations were made from all the embryos. Morphologically and developmentally abnormal embryos were photographed. Slides prepared from chromosomally abnormal embryos were further treated by special banding techniques to identify the extra or missing chromosome.

2. Autoimmunity was induced in female A/J mice by thymectomy at 4-5 weeks of age. At 7 months, these females were mated with young males and chromosome preparations were made on ten to thirteen gestational day embryos. Embryos from sham-operated and non-operated age matched A/J mice were utilized as controls. At the time of sacrifice, sera were taken from each mother for measurement of antinuclear antibody titers.

Major Findings:
1. The mouse has proved to be an excellent model for the maternal age effect with the frequency of aneuploidy increasing from 2.1% in embryos derived from 3-5 month old mothers to 11.1% at 8 to 10 months and 13.0% in embryos derived from mothers over 11 months of age. Both mosaic embryos (with two or more different chromosome complements) and complete aneuploidies (trisomy and monosomy) were observed.

2. The frequencies of chromosomally abnormal embryos among the five inbred mouse strains examined are listed in the following table:

	EMBRYOS DERIVED FROM YOUNG MOTHERS (3-5 MO)			EMBRYOS DERIVED FROM OLD MOTHERS (8- MO)		
STRAIN	MOSAIC >25%	TRISOMIC+ MONOSOMIC	TOTAL ANEUPLOID	MOSAIC >25%	TRISOMIC+ MONOSOMIC	TOTAL ANEUPLOID
C3H/HeJ	1/47	0/47	1/47	6/49	1/49	7/49
A/J	0/45	0/45	0/45	13/84	1/84	14/84
C57B1/6J	1/45	0/45	1/45	2/62	2/62	4/62
CBA/CaJ	1/45	0/45	1/45	4/49	3/49	7/49
NZB	2/57	0/57	2/57	-	-	-
Total	5/229	0/172	5/229	25/244	7/244	32/244

In each of four strains, a definite increase in the frequency of aneuploid embryos was observed as a function of maternal age. Of particular interest was the fact that trisomic and monosomic embryos were only derived from the older maternal age group.

3. The frequency of chromosomally abnormal embryos derived from thymectomized A/J mice was not significantly different from the levels observed in age-matched nonthymectomized controls (17.1% vs 16.7%).

4. A correlation was observed between chromosomal and morphological abnormalities. Aneuploid embryos were frequently grossly abnormal in appearance. Identification of the specific chromosome(s) involved may allow for comparison with the chromosomal abnormalities observed in man as a function of maternal age.

Significance to Biomedical Research and the Program of the Institute: Chromosomal disorders are extraordinarily common in man with a frequency of approximately 1 in 100 live births. This frequency is considerably higher if one considers that over one half the spontaneous abortions that occur during pregnancy are due to chromosomal abnormalities. With increasing maternal age, the risk of having a child with a chromosomal abnormality such as Down's syndrome (mongolism) increases dramatically. A mother at age 45 or above has a 100-fold greater chance of having a child with this syndrome than a mother aged 15 to 20. It is therefore of great clinical importance that insight into the mechanisms of this maternal age effect be obtained.

The mouse has proved to be an appropriate animal model for studying this maternal age effect since a marked increase in the frequency of chromosomally abnormal embryos has been observed with increasing maternal age. A survey of mouse inbred strains did not reveal significant differences in the maternal age effect between strains but instead indicated that the maternal age effect was present to a similar degree in all strains examined. These results suggest a lack of a strong genetic component of the maternal age effect. Similarly, comparison of the frequency of chromosomally abnormal embryos between mice in which immune incompetence was induced and controls did not reveal a significant increase in aneuploidy as a function of altered immunity. Therefore, if the results of these studies can be applied to man, they would suggest that neither genetic predisposition nor altered immunity play a vital role in the increased frequency of chromosomally abnormal offspring born to older mothers.

Proposed Course: The strain survey will be completed by examining the frequency of chromosomally abnormal embryos derived from old NZB mice (a strain with marked immune alterations). Other proposed etiologies for the maternal age such as exposure to X-radiation and infectious agents will be examined. In addition, an attempt will be made to distinguish between uterine and ovarian components of this maternal age effect.

Recent evidence indicates that advanced paternal age may also contribute to the increased frequency of chromosomally abnormal offspring with advanced parental age. This paternal age component will be directly evaluated in human sperm samples derived from volunteer members of the Baltimore Longitudinal Study.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AG-00088-04 LCP

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Mechanisms of Cellular Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. L. Schneider	Medical Officer, PHS	LCP NIA
OTHER:	Y. Mitsui	Visiting Fellow	LCP NIA
	W. H. Adler	Medical Officer, PHS	LCP NIA
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COOPERATING UNITS (if any)

None

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INSTITUTE AND LOCATION NIA, NIH, Gerontology Research Center, Baltimore City Hospitals, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.65	1.3	1.35

SUMMARY OF WORK (200 words or less - underline keywords)

The major objective of these studies is to examine cell replication as a function of aging in cultured human cells in vitro and in vivo in rats and mice. To examine the question of whether age-related alterations seen in vitro reflect in vivo cellular aging, cell cultures established from young and old healthy human donors were examined for their in vitro lifespans and cell replicative capabilities. Significant decreases in fibroblast outgrowth, cumulative cell population doublings and cell population replication rates were found in the skin fibroblast cultures derived from the old donor group when compared to the cultures derived from young donors. Cell separation studies based on differences in cell volumes were initiated in an attempt to obtain cell populations that were enriched for either rapidly replicating cells or slow or nonreplicating cells. These studies successfully isolated out populations of senescent cells whose cell volumes and percent rapidly replicating cells clearly resembled young or early passage cell cultures. However, upon reintroduction of these cells into tissue culture conditions, they rapidly returned to the appearance of senescent cell populations. Further studies are being directed at developing in vivo systems for accurately measuring cell replication.

PHS-6040
(1-75)

Project Description:

Objectives: A decline in the proliferative capacities of certain cell populations is an important feature of human aging. Because of the practical and ethical limitations of in vivo human experimentation, considerable effort has been expended to create appropriate in vitro tissue culture systems as well as in vivo animal models for examining age related alterations in cell replication.

It has been demonstrated that with increasing in vitro age, there is a gradual decline in the frequency of rapidly replicating cells in the diploid fibroblast population. The accumulation of slow or nonreplicating cells results in the diminution of the cell population replication rate which characterizes the senescent phase of the cell population lifespan. Studies were undertaken to isolate from senescent cell populations subpopulations enriched for rapidly replicating cells. These fractionated cell populations would be examined to see if their fractionated characteristics (resemblance to "young" cell populations) could be maintained.

There has been considerable concern expressed over whether in vitro human diploid fibroblast aging reflects in vivo human aging. To examine this important question, cell replicative capacity was measured in a series of human fibroblast cultures derived from old and young healthy volunteer members of the Baltimore Longitudinal Study. In this manner, many of the in vitro aging parameters could be analyzed as a function of in vivo age.

Although in vitro studies of cell replication are important, they are like all in vitro work restricted by the limitations of an artificial environment. It was therefore vital that an in vivo technique be developed to examine cell replicative abilities as a function of age. Previous in vivo studies were performed with radioactive labeling or by examining the end result of cellular proliferation. However, the advent of the new cytogenetic techniques allows for direct examination of cell replication as a function of age without the need for radioisotopes. With these techniques, cell replication will be studied in several animal strains.

Another important aspect of this project will be to examine the ability of cells to repair DNA damage both in vitro and in vivo as a function of age.

Lastly, the relationship between chromosomal alterations (aneuploidy), aging and cell replication will be analyzed by examining cell cultures derived from patients with human genetic disorders, such as the Gardner syndrome, which feature a high frequency of malignant tumors.

Methods Employed:

1. The replicative capacities of skin fibroblast cultures derived from old and young normal volunteer members of the Baltimore Longitudinal Study were assessed by measuring the following parameters: outgrowth of cells from the biopsy tissue, cell population doubling time, cell density at confluency and cumulative cell population doublings.

2. Since previous studies have indicated a close association between cell and nuclear sizes and cellular replicative potential, cell populations were separated by velocity sedimentation to yield subpopulations which differ in cell sizes. The fractions obtained by this technique were then analyzed for cell replicative capability and abilities to maintain their fractionated characteristics.

3. Measurement of macromolecular content as a function of in vitro age was performed by standard Lowry (protein), orcinol (RNA) and diphenylamine (DNA) reactions. Examination of RNA synthesis involved labeling of cells with the appropriate radioisotope precursor (^{3}H-uridine, ^{3}H-methylmethionine) and analysis of the incorporation of this isotope into the major cellular RNA species. Similarly, pulse labeling followed by chasing with the unlabeled precursor for varying time periods allowed for the analysis of the processing and turnover of these RNAs (28 and 18S ribosomal and 4S transfer).

4. For measurement of cell replication in vivo, animals were infused intravenously with BudR. At increasing time intervals, these animals were sacrificed and chromosomal preparations were made. Cells that had undergone 1, 2 and 3 cell cycles in the presence of this drug could be clearly identified by characteristic banding patterns.

5. By utilizing the BudR-differential staining technique both in vitro and in vivo, one can also analyze the frequency of sister chromatid exchanges. This frequency reflects the ability of cells to repair DNA damage.

Major Findings:

1. Skin fibroblast cultures derived from old (ages 65-95) human donors were found to have diminished replicative capabilities when compared to parallel cultures derived from young donors (ages 20-35). Statistically significant differences in cell outgrowth rate, cell population doubling time, cell density at confluency and cumulative cell population doublings were observed.

2. Separation of late passage or "senescent" human diploid fibroblast cultures by velocity sedimentation yielded fractionated subpopulations with marked differences in cell nuclear volumes and in percent replicating cells. In fact, certain fractionated senescent cell populations appeared morphologically identical to early passage cell populations. However, upon reintroduction of these cells to tissue culture conditions, they quickly returned to the appearance of late passage or senescent cells.

3. Investigation of the mechanism responsible for the increased RNA contents of senescent cell populations have indicated that these increases are not due to increased macromolecular synthesis. Further studies will be aimed at examining RNA turnover.

4. Preliminary results of the BudR-differential staining techniques indicate that this technique can be employed to measure cell replication in vivo at levels of BudR that are not inhibitory to cell replication.

5. Measurement of the frequency of sister chromatid exchanges (SCE) in different division cycles produced a ratio of 4.02 first and second division SCE to third division SCE that closely approximates the 4.0 theoretical ratio based on DNA strand polarity.

6. Measurement of SCE frequencies in vivo as a function of BudR dosage revealed that below certain concentrations the frequency of SCE remained stable at 1.5. This strongly supports the spontaneous nature of SCE.

7. In one large family with the Gardner syndrome, we have found a close association between chromosome aneuploidy in cultured lymphocytes and the development of tumors and other aspects of this syndrome. Studies of patients at risk for this syndrome have revealed several individuals with marked increases in chromosomal aneuploidy but without development of the other features of the syndrome.

Significance to Biomedical Research and the Program of the Institute:
Analysis of skin fibroblast cultures derived from young and old human volunteers reveals a decrease in replicative potentials as a function of the age of the cell donor. These results not only support the use of cell cultures to study human aging but also introduce an alternate cell model to early and late passage human fetal lung cell cultures (WI-38) for the study of cellular aging.

Cell separation studies demonstrated the feasibility of obtaining fractionated "senescent" cell subpopulations which greatly resemble unfractionated early passage cell populations. However, the rapid return of these cells to the characteristics of unfractionated senescent cell populations upon return to tissue culture conditions demonstrates the irreversible nature of the loss of "programmed" in vitro replicative capability.

The BudR-differential staining technique has yielded important information of the structure of human DNA and the spontaneous nature of sister chromatid exchanges in human cells.

The finding of increased aneuploidy in a premalignant condition, such as Gardner's syndrome, is an important insight into the relationship between chromosomal imbalance and a genetically programmed human malignancy.

Proposed Course:
The major emphasis will be on the utilization of the BudR-differential staining techniques for the examination of cell replication and repair of DNA damage as a function of age in both in vitro human cell culture and in intact animals.

Studies of macromolecular metabolism (primarily RNA) and karyotypic instability (aneuploidy) will be continued.

Publications:

Stanbridge, E.J., Tischfield, J. and Schneider, E.L.: Appearance of hypoxanthine guanine phosphoribosyl transferase activity in human D98/AH-2 cells: a consequence of mycoplasma contamination. Nature 256: 329-331, 1975.

Tice, R., Chaillet, J. and Schneider, E.L.: Evidence derived from sister chromatid exchanges of restricted rejoining of chromatid subunits. Nature 256: 642-644, 1975.

Schneider, E.L. and Shorr, S.S.: Alterations in cellular RNAs during the in vitro lifespan of cultured human diploid fibroblasts. I. Increased mRNA, rRNA and tRNA content in late passage WI-38 cells. Cell 6: 179-184, 1975.

Schneider, E.L., Mitsui, Y., Tice, R.R., Shorr, S.S. and Braunschweiger, K.: Alterations in cellular RNAs during the in vitro lifespan of cultured human diploid fibroblasts. II. Synthesis and processing of rRNA and tRNA. Mech. Ageing Develop. 4: 449-458, 1975.

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Mitsui, Y. and Schneider, E.L.: Relationship between cell replication and volume in senescent human diploid fibroblasts. Mech. Ageing Develop., in press.

Mitsui, Y. and Schneider, E.L.: Increased nuclear sizes in senescent human diploid fibroblasts. Exp. Cell Res., in press.

Schneider, E.L., Chaillet, J.R. and Tice, R.R.: In-vivo BUdR labeling of mammalian chromosomes. Exp. Cell Res., in press.

Schneider, E.L. and Chase, G.: Relationship between age of donor and in vitro lifespan of human diploid fibroblasts. Interdiscipl. Topics in Geront., in press.

Tice, R. and Schneider, E.L.: In vitro aspects of human genetic disorders which reduce accelerated aging. Interdiscipl. Topics in Geront., in press.

Schneider, E.L. and Mitsui, Y.: Temporal Sequence of "Aging" Parameters in Cultured Human Diploid Fibroblasts. In Proceedings of the 10th International Congress of Gerontology, Vol 1, Plenary Sessions, Symposia. The Congress, Jerusalem, Israel, June, 1975, pp. 69-71.

Schneider, E. and Stanbridge, E.: Comparison of methods for the detection of mycoplasma contamination of cell cultures: a review. In Vitro 11: 20-34, 1975.

Schneider, E.: Detection of Mycoplasma Contamination in Cultured Cells: Comparison of Biochemical, Morphological and Microbiological Techniques. In Prescott, D.M. (Ed.): Methods in Cell Biology. New York, Academic Press, 1975, Vol X, pp. 261-275.

Schneider, E. and Stanbridge, E.: A Simple Biochemical Technique for the Detection of Mycoplasma Contamination of Cultured Cells. In Prescott, D.M. (Ed.): Methods in Cell Biology. New York, Academic Press, 1975, Vol X, pp. 277-290.

PROJECT NUMBER (Do NOT use this space)

HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

Z01-AG-00089-02-LCP

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Human Immunology Program

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. M. B. Kay	Medical Officer, PHS	LCP NIA
OTHER:	T. Makinodan	Chief, LCCP	LCP NIA
	S. Hausman	Staff Fellow	LCP NIA
	S. Sterioff	Asst Chief of Surgery	Balto City Hosps.
	H. Fudenberg	Professor	Med Univ of S.C.

COOPERATING UNITS (if any)

Renal Transplantation Service, Baltimore City Hospitals, Baltimore, Maryland
Dept of Basic & Clinical Immunology & Microbiology, Medical University of South Carolina, Charleston, South Carolina

LAB/BRANCH

Laboratory of Cellular and Comparative Physiology

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Gerontology Research Center, Baltimore City Hospitals, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.4	0.9	1.5

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to determine which immune indices, measured predominately by in vitro assays, correlate best with in situ immune function, and to miniaturize these assays. The topics presented include (1) optimal culture conditions for PHA response, (2) comparative studies on human spleen, lymph nodes, and peripheral blood, (3) comparative studies between immunologic parameters of human and mouse lymph nodes and spleen, and (4) studies on peripheral blood and splenic cells from patients with hairy cell leukemia.

Project Description:

Objectives: The immune system is perhaps the most promising of those being investigated by gerontologists for it is the best defined system to study cellular and molecular aging. Further, a causal relationship between its integrity and susceptibility to disease is suggested by numerous studies. Thus, as normal immune functions decline with age, the incidence of infections, autoimmune disease, and cancer increases.

The purpose of this project is to determine which immune indices, measured predominantly by in vitro assays, correlate best with in situ immune status and to miniaturize these assays so that they can be performed on relatively small numbers of cells. The validity of these studies are being assessed by parallel cross-sectional studies of eight strains of mice in five age groups.

Since studies on human immune indices are performed on peripheral blood cells, a part of this project will be directed toward determining whether circulating lymphocytes are representative of lymphocyte populations in antigen processing centers, such as lymph nodes and spleen.

Methods Employed: In this study, peripheral blood (PB) was obtained from healthy individuals (i.e., individuals without evidence of any organ or system disease) over age 21 in order to further develop and miniaturize techniques and methodologies for assaying human T and B cell function prior to undertaking a longitudinal study.

Both T and B cells indices were tested. The assays for B cell function included: Ig levels, mitogenic response to pokeweed, and determinations of the number of circulating B cells using immunofluorescence and the rosette techniques. Assays for T cells included determination of the number of circulating T cells using fluorescent anti-thymocyte serum and the nonimmune rosette technique, mitogenic response to phytohemagglutinin (PHA), skin test response to intermediate strength tuberculosis, mumps, streptokinase and/or candida skin test antigens or DNCB, and mixed lymphocyte cultures.

For establishing PHA dose response curves relative to T cell proportion, anti-Fab columns are utilized for cell separation, after which T and B cells are recombined in known proportions between 0 and 100%. The response of peripheral blood, spleen, and lymph node cells from trauma victims (most of whom died of head injuries) whose organ perfusion is maintained until organ removal for renal transplantation, and spleens from healthy donors which are removed because of aneurysms of the splenic artery, are compared by the same techniques. In addition, assessment of organ cellularity (cells/g) and cell viability are performed. Mouse experiments included the same assays as those performed on human lymph nodes and spleens as well as assessment of age effect on colony-forming ability of stem cells.

Major Findings:

1. Kinetic and cell concentration studies on human peripheral blood indicate that the optimal cell concentration in a microculture system using round-bottomed culture plates (the smallest wells that can be used and still permit utilization of an automatic harvester) is between 2.5 and 10 X 10^4 cells in 100µl of medium per well, and that the optimal pulse time is between 90 and 118 hours using a pulse duration of 16 hour and 12 hour pulse intervals. There is no significant difference between response to PHA doses between 0.03-3µg.

2. Macrophage depletion appears not to adversely affect the PHA response nor alter the dose response curve. In fact, results suggest that macrophage depletion enhances the response.

3. Studies in progress on spleen, lymph node and peripheral blood (13 individuals studied to date) indicate that the (a) peripheral blood PHA dose response is comparable to that of lymph nodes and spleen; (b) removal of polymorphonuclear leukocytes and red blood cells increases the response of spleen cells but has a minimal effect on lymph node cells; (c) optimal cell concentration of lymph node and spleen cells is similar to that of PB except that good responses can be achieved at cell concentrations of 5 X 10^3 in 100µl per well with a few individuals tested; however, there is great variability between an individual's ability to respond at this cell concentration; (d) storage of human tissue on ice for 12-24 hours (which is often necessitated by conditions of removal and transport) significantly decreases the PHA response at all doses by increasing the background by 2-3 $\log_{10}$ in the macro culture system which utilizes 2.5 X 10^5 cells/200µl in flat-bottomed tissue culture wells. This elevation of background is less pronounced in the microculture system where the background is increased by 1-2 logs; (e) enrichment of T cells to approximately 100% increases the PHA dose response by 0.5-1 log over Ficoll-Hypaque separation alone; whereas T cell depletion (essentially pure B cell preparation) reduces the response to 0 (2 experiments).

4. Comparison between immunologic parameters of human and mouse lymph nodes and spleens indicates that the: (a) percentage of T and B cells in human and mouse lymph nodes and spleen is similar; (b) response of lymph node cells to PHA is greater than the response of spleen cells to PHA; (c) response of spleen cells to PHA decreases with increasing age while the response to PW or LPS increases relative to the PHA response; (d) decrease in PHA response with age is due to an increase in the background. This suggests that more cells are turning over because of cell death. This same phenomena of increased background is seen in human spleens and lymph nodes kept on ice for 24 hours before processing.

5. Studies on PB and spleen cells from individuals >45 years of age with "hairy cell" leukemia suggest that the leukemic cells are either B cells or plasma cells.

Significance to Biomedical Research and the Program of the Institute: It is anticipated that research on the cellular and molecular pathogenesis of age-associated diseases will permit the formulation of approaches to minimizing the debilitative processes associated with aging.

Proposed Course: Present studies will be extended and expanded. Cell concentrations and kinetic studies including determining the optimal pulse concentration and interval in the microculture system will be refined. In vitro response to antigens such as PPD, sk-sd, etc., will be assessed. We will attempt to modify the following assays so that they can be used as in vitro tests for human lymphocytes:

1. The plaque forming cell assay (Jerne and Nordin, Science 140: 405, 1963; Mishell and Dutton, J. Exp. Med. 126: 423, 1967; Lemieux, S., Avrameas, S. and Bussard, A.E., Immunochem. 11: 261, 1974).
2. "Helper" T cell function assay using the method of Farrar (Farrar, J.J., Loughman, B.E. and Nordin A., J. Immunol. 112: 1244, 1974).

Publications:

Kay, M.M.B.: High Resolution Scanning Electron Microscopy (SEM) and its Application to Age-related Changes of T and B Cells. In Proceedings of the 10th International Congress of Gerontology, Vol. 1, Plenary Sessions, Symposia. The Congress, Jerusalem, Israel, June 1975, pp. 84-86.

Kay, M.M.B.: Surface characteristics of Hodgkins cells. Lancet II: 459-460, 1975.

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Kay, M.M.B.: High Resolution Scanning Electron Microscopy and its Application to Research on Immunity and Aging. In Makinodan, T. and Yunis, E. (Eds.): Immunology and Aging. New York, Plenum Press, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-AG-00090-02-LCP

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Membrane Differentiation: Effect of Aging on Modulation of Receptor Formation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. M. B. Kay	Medical Officer, PHS	LCP NIA
OTHER:	T. Makinodan	Chief, LCCP	LCP NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Cellular and Comparative Physiology

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INSTITUTE AND LOCATION NIA, NIH, Gerontology Research Center, Baltimore City Hospitals, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	0.3	1.0

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to determine the effect of aging on cell membrane receptors, function, and integrity. Parameters of membrane integrity were studied using cells from young or old mice before and after a Sendai infection of the GRC animal colony. It was found that (1) cells from old mice were more fragile than those from young mice before the Sendai infection, and (2) that Sendai infection increased the frequency of cell death at both 4°C and 37°C; however, the frequency of cell death in cell populations from old mice was 50% higher than in those from young mice. Lymphocytes from elderly humans were found to be more fragile than those from young individuals.

PHS-6040
(1-75)

Project Description:

Objectives: Immunologic activities generally decline as an individual ages. Since the early events in antigen activation occur at the level of the cell membrane, the effect of aging on membrane receptors, function and integrity were investigated.

Methods Employed: Splenic cells from young and old mice were separated into subpopulations by their differences in density, adherence to nylon wool, and differential susceptibility to antisera reagents. The subpopulations were viewed with scanning electron microscopy following each treatment. Human lymphocytes were separated into T and B cell populations using the SRBC rosette technique followed by density separation on Ficoll-Hypaque. Subpopulations on B lymphocytes were identified by the use of antisera reagents (anti-human IgA, M or G) conjugated to scanning electron microscopy visible markers. Varying osmolarities (in 10% steps from 0 to 100% of the osmotic pressure of physiologic solutions) were used as a probe of cell fragility.

Major Findings:

1. Peripheral blood lymphocytes from elderly individuals (more than 60 years old) were more fragile when tested with solutions of varying osmolarity than cells from young adults (20-25 years of age). T cells were more fragile than B cells.

2. Lymphocytes from lymph nodes, thymii, and bone marrow of old mice were more fragile (i.e., showed a high frequency of cell death) than cells from young mice during routine manipulation for tissue culture.

3. Following a natural Sendai virus infection of the GRC animal colony, the frequency of cell death during routine manipulation for tissue culture increased by approximately 20% in all tissues.

4. Following the Sendai infection, the frequency of cell death after a 30 minute incubation at 37°C was 50% greater than for cells from old mice than for those from young mice. Thus, in vitro culture techniques do not provide valid comparisons when Sendai is present in an animal colony.

Significance to Biomedical Research and the Program of the Institute: The results of these studies are vital to our understanding of aging at a cellular level, and will form the basis of studies directed toward reconstitution of immune function in the aged.

Proposed Course: Project terminated because of Sendai infection of the animal colony, including experimental and aging rooms, in January, 1976.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-AG-00091-02-LCP

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Mechanism of Removal of Senescent Cells by Human Macrophages In Situ

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. M. B. Kay	Medical Officer, PHS	LCP NIA
OTHER:	S. Oh	Visiting Associate	LCP NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Cellular and Comparative Physiology

SECTION

INSTITUTE AND LOCATION NIA, NIH, Gerontology Research Center, Baltimore City Hospitals, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0.0

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to determine the mechanism by which macrophages distinguish between adult and senescent self. Experiments indicate that human macrophages make this distinction on the basis of selective IgG attachment to the surface of senescent cells. The objective of this project is to isolate and characterize the regulatory IgG. The antibody eluted from senescent cells was shown to be (1) an IgG without other immunoglobulins by immunodiffusion, immunoelectrophoresis, and acrylamide gel electrophoresis, (2) polyclonal, and (3) a regulatory autoantibody.

PHS-6040
(1-75)

Project Description:

Objectives: Macrophages distinguish between "self" and "nonself;" that is, they phagocytize foreign cells while leaving their own alone. Since they phagocytize senescent self cells, they must also be capable of distinguishing between "mature self" and "senescent self." Experiments indicate that macrophages make this distinction on the basis of selective IgG attachment to the surface of senescent RBC (Kay, 1975). The nature of the IgG and the receptor to which it binds are, as yet, unknown. The objective of this project is to isolate and characterize the regulatory IgG, presumably an autoantibody, and its receptor in order to determine (1) subclass, (2) binding specificities, (3) complement binding properties, and (4) T cell dependency.

Methods Employed: Initially, the human RBC system will be utilized as a model because (a) macrophages routinely phagocytize RBC at the end of their 120 day lifespan and (b) in many respects, it is an "ideal" system with which to work (i.e., large numbers of cells are readily available and easily isolated, the membrane has been extensively characterized biochemically and RBC have a smooth regular surface which does not "cap" or ingest labels). Two biological assays are readily available: a phagocytosis assay which assesses macrophage recognition of senescent cells (Kay, 1974; Kay, 1975) and scanning electron microscopy (SEM) labeling technique which assesses IgG attachment, density and distribution (Kay, 1975).

Major Findings:

1. The regulatory autoantibody has been isolated from the freshly drawn RBC of five individuals and has been shown by immunodiffusion (ID), immunoelectrophoresis (IEP) and acrylamide gel electrophoresis to be an IgG free of other serum components and immunoglobulins.

2. The IgG appears to be polyclonal by IEP and reacts (stongly) with anti-λ and (weakly) with anti-K in ID and IEP.

3. The IgG eluted from RBC when incubated with in vitro aged autologous RBC initiates their phagocytosis by autologous macrophages.

4. Allogeneic autoantibody initiates phagocytosis of in vitro aged RBC by autologous macrophages.

5. The IgG eluted from in situ aged RBC initiates phagocytosis of neuraminidase-treated young RBC.

Significance to Biomedical Research and the Program of the Institute: The results of these experiments are vital to our understanding of the mechanism by which senescent cells are removed and suggest that receptor molecules on cell membranes "age."

Proposed Course: As described. Binding studies and isolation and characterization of the RBC receptor are in progress.

Publications:

Kay, M.M.B.: Mechanism of removal of senescent cells by human macrophages *in situ*. Proc. Nat. Acad. Sci. 72: 3521-3525, 1975.

Kay, M.M.B.: Multiple Labeling Technique for Immuno-scanning Electron Microscopy. In Hayat, M.A. (ed.): Principles and Techniques of Scanning Electron Microscopy. New York, Von Nostrand Reinhold Co., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-AG-00092-04-LCP

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Cellular Aspects of the Immune Response of Long-term Radiation Induced Allogeneic Chimeras

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Albert A. Nordin Research Chemist LCP NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH
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INSTITUTE AND LOCATION NIA, NIH, Gerontology Research Center, Baltimore City Hospitals, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this project are to study the immunological problems resulting from the transplantation of allogeneic bone marrow cells. The problems encountered in successfully transplanting allogeneic bone marrow cells as well as the immunological defects resulting after the graft is established are under investigation. Lethally irradiated mice grafted with allogeneic bone marrow cells serve as the model for these investigations.

PHS-6040
(1-75)

Project Description:

Objectives: The major objective of the proposed research is to examine the immune system of x-irradiated allogeneic bone marrow grafted mice. These allogeneic radiation chimeras serve as a model for investigating the basic cellular aspects of immune mechanisms with particular emphasis on regulatory mechanisms involved in immunologic responsiveness.

Methods Employed: Previous work has established the fact that allogeneic bone marrow chimeric mice can only survive the secondary disease effects of a graft-versus-host (GVH) reaction in a controlled germfree environment. However, there is reasonable evidence to suggest that the clean environment provided by a laminar flow animal facility will also promote long-term survival. Animals quartered in a laminar flow room are given a low lethal dose of x-irradiation followed by a bone marrow graft from either a syngeneic or allogeneic donor. Subsequently observed immunologic deficiency will be examined to determine the cellular aspects of the deficiency. Experimental attempts to compensate for or eliminate the deficiency will be carried out.

Major Findings: Allogeneic bone marrow chimeric mice develop a deficiency in the B-derived lymphocyte population that is expressed only after the mice have aged. This deficiency was of the order of 50% when compared with age matched normal mice or with syngeneic bone marrow chimeric mice. This reduced responsiveness to a T-independent antigen was observed both *in vivo* and *in vitro*.

The known functional T-cell deficiency of allogeneic bone marrow chimeras was substantiated by a low or nonexistent response to T cell mitogens, PHA and Con A. Although there are approximately 10% theta-positive cells in chimeric spleens, this population is not reactive to these mitogens. However, an aliquot of the same spleen cell suspension is as active and in some instances more active than the controls when tested in a MLC. Further studies showed that the MLC reactivity did not correlate with the generation of cytotoxic killer cells; i.e., ^{51}Cr release assays showed no reactivity of chimeric spleen cells.

Significance to Biomedical Research and the Program of the Institute: The results of these investigations will be significant in a practical sense. Clinical bone marrow grafting within a heterogeneous population has necessarily been restricted mainly as a result of immunologic damage to the host. These results with chimeras maintained in the laminar flow environment resemble the results seen in human bone marrow grafting. Information may then be available which will reduce the risks in bone marrow grafting and result in the reconstitution of an intact immune mechanism.

Basic information pertinent to the immune response is equally significant. Cellular immunology, during recent years, revolved around cell cooperation as a basic requisite for the expression of antibody to many antigens. The information which can be supplied in attempts to re-establish immunological competence to the allogeneic chimeric mice is expected to

demonstrate and define the importance of cellular interactions in both the humoral and cell-mediated forms of the immune response.

Studies concerned with immunodeficiency diseases will be of significance since many naturally occurring examples of such diseases have traced a causative factor(s) to the thymus gland. This model system with a well defined thymic defect should provide meaningful data relevant to the immunodeficiency disease state.

Proposed Course: This project will be terminated.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-AG-00093-04-LCP

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Cellular Basis of Regulation of the Humoral Immune Response

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Albert A. Nordin Research Chemist LCP NIA

COOPERATING UNITS (if any)

None

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Laboratory of Cellular and Comparative Physiology

SECTION

INSTITUTE AND LOCATION NIA, NIH, Gerontology Research Center, Baltimore City Hospitals, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.75	0.6	1.15

SUMMARY OF WORK (200 words or less - underline keywords)

The regulatory role of lymphoid cells and their products on the humoral immune response is the intent of this project. The regulatory mechanisms are being investigated in vitro using a T-cell dependent as well as a T-cell independent antigen. The cellular requirements and functions involved in the in vitro immune response are being established for normal adult mice. These are then being compared to those in aged mice of the same strains. The possibility that regulatory mechanisms observed in young adult mice are amplified in old mice resulting in immunosenescence is being investigated.

PHS-6040
(1-75)

Project Description:

Objectives: The goal of this project is to characterize the cells regulating the immune response by cellular elements in both young and aged mice. Efforts to determine the origin and mechanism of action of these cells are of prime interest.

Methods Employed: (1) The in vitro culture techniques and the assay for plaque-forming cells are routine methods.

(2) Carbonyl-iron treated spleen cells is performed by adding 25 mg of sterile carbonyl iron to 100 x 10^6 normal spleen cells. After a 30 minute incubation at 37°C in a 5% CO_2 environment, the iron and cells with ingested iron are removed by magnetic attraction. This process is repeated and the spleen cells free of iron-ingesting cells is used as a source of T and B lymphocytes.

(3) Peritoneal exudate cells are collected from unstimulated mice and used as a source of accessory cells. These cells are either used directly or an adherent layer prepared from them before other cell types are added.

(4) DAGG-Ficoll is prepared by modifying Ficoll by introducing carboxyl-methyl amino-ethyl groups to which is added the tri-peptide glycine-glycl-alanyl with the terminal alanine substituted with a single dinitro phenol haptenic group. The preparation used here contains 48 moles of hapten per mole of Ficoll.

Major Findings: The antibody response of old (22-24 month) C57BL/6 mice was studied in vivo and in vitro to a T-independent antigen, DAGG-Ficoll. The in vivo response clearly showed that there was an intact response to this antigen in most old mice. The variation between individuals of the old population is larger than that seen in the young population. The variation observed in the old mice was however not due to an antigen limiting effect. The antigen dose was raised 10-fold without effecting the level of the response or reducing the variation. Although there was some gross pathology (tumors, cysts, splenic abnormalities, etc.) seen in 10% of the old mice, there was no consistent effect on the immune response.

The antibody response as observed in vitro to the same antigen in similar aged mice was also investigated. From the initial studies it was very obvious that a pool of spleen cells from old mice does not accurately represent the response of the individuals in the pool. In each instance the response of the pool is considerably lower than the average response of the individuals. As a result, 15 individual old mice were assayed separately. Unlike the results in vivo, the in vitro system showed a significantly lower response of the old mice as compared to the young mice. It is significant to note that the B cell proliferations as indicated by the response to this antigen is not a general indices of B cells in total since in some mice the response to sheep erythrocytes was markedly increased over that to the T-independent antigen.

The studies on the presence of a naturally occurring lymphoid cell population that regulates the in vitro antibody response have shown that the second adherent layer prepared from normal spleen contains the suppressor population. This population of cells was left adherent to the culture vessel and supplemented with a purified source of T and B derived lymphocytes and an accessory cell source. Such culture conditions result in nearly a total suppression of the antibody response to sheep erythrocytes.

It became apparent during the course of these studies that investigations on the regulation of the immune response as studies in vitro require a constant source of active accessory cells. The accessory cell activity is most likely the initial cellular event in the immune response and any malfunction of this population can drastically reduce the immune response. As a result a suppressive effect seen in in vitro cultures can be due to this population and not to T or B derived lymphocytes. This is further complicated since normal spleen is not a consistent or reliable source of accessory cells. In our hands, the adherent layer prepared from normal peritoneal exudates is most consistent and the activity of these cells can be titrated.

Significance to Biomedical Research and the Program of the Institute: The goal of this research program is to examine the cellular populations that are regulating the humoral immune response. The mechanisms by which the regulation takes place would be of significance not only to the field of immunology but would have relevance to cell biology. It is also significant to the area of immunosenescence. The decline in immunological responsiveness with age is well established but the reasons are not at all understood. The role of regulatory mechanisms in explaining the phenomena of immunosenescence may be of considerable significance.

Proposed Course: (1) Attempt to explain the observation made in old mice that the response to a T-independent antigen results in a lower response than that to a T-independent antigen.

(2) Attempt to characterize the nature of the in vitro response of old C57Bl/6 mice to DAGG-Ficoll by adopting a micro-culture technique. This would permit (a) more extensive assay, (b) more combinations of the various cellular elements and (c) more definitive studies on the reasons underlying the reduced in vitro response.

(3) Attempt to determine the frequency of antigen specific cells and their burst size in old C57Bl/6 mice.

(4) Attempt to determine the mechanism of regulation of adherent suppressor cells and then to examine old C57Bl/6 mice for such a population.

Publications:

None

[illegible] PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-AG-00094-03-LCP

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Characterization of Immune System of Aging Mice with Immunodeficiency (Diseases)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. Hirano	Visiting Fellow	LCP NIA
OTHER:	A. A. Nordin	Research Chemist	LCP NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Cellular and Comparative Physiology

SECTION

INSTITUTE AND LOCATION NIA, NIH, Gerontology Research Center, Baltimore City Hospitals, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	0.0

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to characterize the in vitro cell-mediated immunity in young and aging mice of various genetic strains. The relationship between the immunodeficiency in aging and the regulation, in the form of suppression of the immune system, is investigated. The topics of present interest are: (1) the regulatory mechanisms of the in vitro development of cytotoxic lymphocytes by suppressor cells, (2) the characterization of suppressor cells, and (3) the characterization of the immunodeficiency in aging and its relationship with suppressor cells.

PHS-6040
(1-75)

Project Description:

Objectives: The goal of this project is to characterize the immune system in young and aging mice of various genetic strains. The relationship between the immunological disorders and the regulation, in the form of suppression of the immune system, is investigated.

Methods Employed: (1) A modification of the spleen cell culture system of Mishell and Dutton is used. Spleen cells from individual mice or pooled spleen cells are cultured with mitomycin-C treated or irradiated allogeneic spleen cells, F_1 spleen cells or heterologous erythrocytes at 37° C for various days. The double chamber culture system is also used.

(2) Cytotoxicity assay - ^{51}Cr labelled EL-4 or P-815 cells are mixed with cultured spleen cells and incubated for various times. After incubation, cold PBS is added, the tubes centrifuged and the radioactivity of the supernatant counted.

(3) Plaque-forming cell assay-routine technique used to detect IgM and IgG antibody-producing cells.

Major Findings: (1) Under stimulation by alloantigen, cytotoxic lymphocytes (CL) develop and at the same time suppressor cells also develop in mixed lymphocyte culture (MLC).

(2) Suppressor cells are anti-theta sensitive and derived from the cortisone sensitive spleen cell population.

(3) A suppressor cell population when separated from a responsive MLC by a nucleopore membrane, pore size 0.20μ, effectively suppressed the development of CL.

(4) Suppression was observed only when the suppressor cells were restimulated with the same H-2 type cells used for induction.

(5) The suppressive effect was H-2 non-specific; i.e., $H\text{-}2^b$ suppressor cells induced by $H\text{-}2^k$ cells suppressed the development of CL by $H\text{-}2^b$ cells to $H\text{-}2^d$ cells. In addition, $H\text{-}2^b$ suppressor cells effectively suppressed the development of CL from $H\text{-}2^k$ cells.

(6) Suppressor cells could be induced by H-2 region differences alone; i.e., B10($H\text{-}2^b$) spleen cells stimulated by B10·D2 ($H\text{-}2^d$) or B10·BR ($H\text{-}2^k$) effectively suppressed the development of CL.

(7) The suppressive effect was not due to a non-specific cytotoxic activity.

(8) The development of CL decreased in aged NZB, DBA/2 and C57Bl/6 mice.

(9) The decline in the development of CL in aged C57Bl/6 mice was inversely correlated with the development of suppressor cells; i.e., CL developed very well from the mice which showed low suppressor cell

development whereas very low levels of CL developed from the mice which showed high levels of suppressor cells.

Significance to Biomedical Research and the Program of the Institute: This proposal offers two main significant contributions: (1) the regulatory mechanism of cell-mediated immunity in young and aged mice, and (2) the mechanism of the immunosenescence of cell-mediated immunity.

Proposed Course: The regulatory mechanism(s) in cell-mediated immune response in young and aged mice will be established.

Publications:

Hirano, T. and Nordin, A.A.: Cell-mediated immune response in vitro. I. The development of suppressor cells and cytotoxic lymphocytes in mixed lymphocyte cultures. J. Immunol. 116: 1115-1122, 1976.

Hirano, T. and Nordin, A.A.: Suppressor cells in cell-mediated immunity. J. Immunol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-AG-00095-03-LCP

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

The Role of Cell Membrane Structures on Cellular Recognition

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. H. Adler	Medical Officer, PHS	LCP NIA
	H. Nariuchi	Visiting Fellow	LCP NIA
	K. H. Jones	Staff Fellow	LCP NIA
	J. W. Heine	Staff Fellow	LCP NIA
	M. A. Brock	Research Biologist	LCP NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Cellular and Comparative Physiology

SECTION

INSTITUTE AND LOCATION NIA, NIH, Gerontology Research Center, Baltimore City Hospitals, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.2	2.95	1.25

SUMMARY OF WORK (200 words or less - underline keywords)

The correlation of morphologically distinct immunocytes with a specific set of functions is necessary in order to evaluate age associated immunodeficiency. Use of an anti-B cell antisera has shown that the spleens from old mice, while performing well in a "B" mitogenic assay, actually do not have normal B cells. Several B cell criteria change with age and quantitation of B cells must be done carefully. Studies in the whole animal have shown a decreased tumor immunity along with a poor T cell function which may be able to be correlated.

Project Description:

Objectives: To correlate certain immunologic functions with morphologically identifiable populations of immunologically active cells. The functional criteria and results will be compared to arrive at correlations in order to arrive at methods for diagnosing and describing immune deficiency and assigning certain predictive projections of immune function. The tests of immune functions will include responses to mitogen and antigen in in vitro culture conditions, responses to antigens and oncogenic stimuli in vivo, and the development of immunologically competent cells in in vitro environments. These studies will give a better understanding of age related immunodeficiecy.

Methods Employed: The basis of most functional assays will be in vitro culture systems. There will be both short term for the investigation of mitogen and antigen responsiveness and for the generation of antibody forming cells, and longer time for the generation of cytotoxic lymphocytes and antibody forming cell colonies. In vivo methods will primarily be cell transfer studies and transplantation studies with syngeneic tumor cells. The cells will be from various lymphoid organs and from varying aged donors. The cells will be treated by physical separation methods and with specific antisera to eliminate certain populations, or to quantitate various cellular population representation.

Major Findings: There are many difficulties with manipulative approaches to age-associated disorders of immune function, the primary one being the nature of the aging process itself. Aging must be viewed as a normal, pathogenetic process; but when does the process start? At what point can you assay an immune response and say that it represents an aging effect--or a lack of an aging effect? If one must wait until the appearance of an age-related disease to show a decrease in immune function, then one must deal with pathogenetic factors in addition to aging which might effect declining functions of the immune system. Therefore, from the very basic problems in deciding what constitutes an age-related immunodeficiency, there must be further consideration of what assay to use, what to infer from the results of the assay, what to predict in terms of function in other systems and what a deficiency might mean in terms of disease susceptibility.

The methods utilized for a short term lymphocyte culture system for demonstrating a moderate level of immunodeficiency are extremely important. The culture system assayed at certain times will show that the cells from the deficient animal will function at a better than normal rate. Also at certain cell densities in culture the deficient cells will perform at a better than normal level.

In studying various immunodeficient mice it was found that, although some decreased T cell functions can be correlated to a low level of T cells present, others can not. While a PHA response may be low, a mixed lymphocyte response may be normal and while an MLR may be normal the generation of cytotoxic cells may be low.

The response to mitogen of lymphoid tissue from old mice of one inbred strain shows an individualistic pattern of response and the pattern of response of each tissue was independent of any other lymphoid tissue in the same mouse. As such, mitogen responsiveness per se may not be a useful diagnostic or descriptive tool for determining an immune deficiency and each functional test must be evaluated on its own. Until we have better tests it is not possible to make assumptions based on one test of the cellular functional capacity in a different assay.

In old mice, the spleen and thymus can show a marked responsiveness to the mitogen endotoxin LPS. In young mice the cells responding are primarily B lymphocytes but in the old mice the responding cells do not possess some of the morphologic criteria of a B cell, nor do they respond to B cell antigen to develop into antibody forming cells. Therefore, the response to a B cell mitogen in the old mice is not correlated to the presence of B cells or to a functional B cell activity.

Lymphoid spleen cells from old mice are not selectively more sensitive to physical damage, so that the preparation of cell suspensions from the old spleens does not result in a selection of cell types due to a selective loss.

Using the immunoglobulin (Ig) surface marker or a "B" cell criteria under the usual circumstances is not valid in studying the older spleen. Many Ig positive cells are not B cells.

An antisera has been developed which has specificity against an immature B cell and stem cells. The antisera will destroy colony forming cells, LPS reactive cells, and sheep cell antibody forming cell precursors, but will not kill plasmacytoma cells, or antibody forming cells. Together, with an in vitro culture system for growing B cell colonies in agar, it is possible to trace the differentiation of B cells from stem cells to antibody forming cells.

Methylcholanthrene sarcomas in 3 inbred strains of mice have been characterized and shown to be antigenic and immunogenic. Smaller cell numbers are needed to induce tumors in an aged recipient, however, these studies have given inconsistent results. This is primarily because the tumor line becomes infiltrated with host cells; up to 50% of the cells in a tumor can be normal host cells. Therefore, the inoculum can vary as to the number of tumor cells, and the normal cells can contribute to the host resistance to challenge with tumor. Furthermore, the tumors in the old animals vascularize poorly and a failure of growth may not reflect an immune reaction. To solve these difficulties it is necessary to pass the tumors, prior to challenge, in an F-1 host and use allo-antisera to eliminate normal host cells. The challenge then is carried out in young mice with an attempt to neutralize with lymphoid cells from old and young immune cell donors.

Techniques have been developed to allow freezing of immune cells with subsequent recovery of 70-90% of control levels of functions. This will allow certain tumor experiments to be carried out on mice throughout

their life, using the same mouse as the recipient of its own immune cells together with its own tumor, at various ages to determine therapeutic benefits of stored anti-tumor-immune cells.

Significance to Biomedical Research and the Program of the Institute: We are gaining a better definition and appreciation of the term immunodeficiency. Since a relative immunodeficiency is seen in aging, it is important to develop better diagnostic criteria, so that possible remedial measures can be undertaken.

Proposed Course: To continue to outline the connection between form and function and to expand our technical ability to measure functions. To develop better diagnostic criteria and tests to describe immune capacity.

Publications:

Adler, W.H.: Aging and immune function. BioScience 25: 652-657, 1975.

Ozato, K., Adler, W.H. and Ebert, J.D.: Synergism of bacterial lipopolysaccharides and Concanavalin A in the activation of thymic lymphocytes. Cell. Immunol. 17: 532-541, 1975.

Ozato, K., Ebert, J.D. and Adler, W.H.: Pretreatment of murine thymocytes by PHA inhibits bonding of ^{3}H-Concanavalin A. J. Immunol. 115: 339-344, 1975.

Ozato, K., Adler, W.H. and Ebert, J.D.: The differentiation of suppressor cell populations as revealed by studies of the effects of mitogen on the mixed lymphocyte reaction and on the generation of cytotoxic lymphocytes. Cell. Immunol., in press.

Spero, L., Leatherman, D. and Adler, W.H.: Mitogenicity of formalinized toxoids of staphylococcal enterotoxin B. Infection & Immunity 12: 1018-1020, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-AG-00096-03-LCP

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Low Temperature Effects on Cells of Aging Individuals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. A. Brock	Research Biologist	LCP NIA
OTHER:	W. H. Adler	Medical Officer, PHS	LCP NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Cellular and Comparative Physiology

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INSTITUTE AND LOCATION NIA, NIH, Gerontology Research Center, Baltimore City Hospitals, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this study are to characterize possible age-related differences in the in vitro responses of murine lymphohemopoietic cells to mitogens and their differential susceptibility to freezing damage. Current work focuses on the assessment of functional capacity in the in vitro environment where conventional culture methods may not accurately reflect true functional capacity. Augmented "responses" to all the tested mitogens were observed by raising ^{3}H-thymidine concentrations, reflecting what could be interpreted as increased DNA synthesis and cell proliferation. Enhancement of ^{3}H-thymidine incorporation was observed in different strains of mice and in both young and old mice.

PHS-6040
(1-75)

Objectives: To characterize the functional capacity and structure of pre- and post-mitotic cell types from aging individuals, specifically the possible age-related differences in (1) the in vitro responses of human and murine lymphohemopoietic cells to various agents and (2) the differential susceptibility of lymphohemopoietic cells to freezing damage assessed by their functional recovery after thawing.

Methods Employed: The incorporation of H^3-thymidine into lymphocytes from mouse splenic cell suspensions was used as an indication of DNA synthesis and cell replication in vitro. The T-cell mitogens, PHA and Concanavalin A, the B-cell mitogen, LPS, several types of substrates and alcohols were added with H^3-thymidine to different experimental cultures.

Major Findings: The lymphocytic responses to at least three different mitogen concentrations as a function of H^3-thymidine concentration were further characterized. A marked enhancement in the incorporation of thymidine had been observed and was directly related to H^3-thymidine concentration from 0.5 to 5.0 μC per culture, using thymidine with a specific activity of 6.0 Ci/mM. A similar rise in H^3-thymidine incorporation with increasing concentration was also observed using a lower specific activity isotope, 2 Ci/mM. The possible roles of various substrates, such as glucose and phosphorylated sugars, and alcohols except for ethanol in inducing the augmented responses were eliminated. Appropriate controls showed that the isotope was indeed intracellular. Therefore, it is possible to demonstrate what appears to be increasingly greater DNA synthesis and cell replication in C57Bl/6 mice simply by raising the H^3-thymidine concentration. There was a similar effect in the DBA/2 mouse strain, but with differences in the absolute amount of H^3-thymidine incorporated that depended on the stimulating mitogen. For example, using the mitogen, Concanavalin A, and increasing H^3-thymidine from 2.5- to 5-fold resulted in a rise in thymidine incorporation of 3.6 and 6.3 times the basal values in DBA/2 mice but only 1.9 and 2.1 times in C57Bl/6 mice. In preliminary experiments with 2-year-old C57Bl/6 mice, a similar increased incorporation of thymidine with rising H^3-thymidine concentration was observed. In fact, the enhancement of the responses could be greater in the older animals than in young mice. If the actual amounts of bound H^3-thymidine are considered, these could rise in the older mice to levels above baseline levels of young mice.

A seasonal decline in the in vitro response of murine lymphocytes to T-cell mitogens was observed in a second year. A fall in the immune response as assessed by plaques formed in vitro following in vivo antigen stimulation has been reported in ground squirrels kept in natural lighting conditions during the winter months. In the present studies, lymphocytic responses to PHA, LPS and Concanavalin A in vitro were monitored in cells from mice in a constant temperature and 12:12 LD environment. The results suggest that the seasonal decline in immune response is endogenous.

Modifications of the Linde BF4 Biological Freezing System have included reconstruction of the controller unit to provide a constant rate of cooling from 0.1 to 10.0° C./min. controlled by an electronic ramp.

Delays of over 6 months in procuring electronic components have prevented the changes necessary to control the variability in cooling rate at the point of freezing.

Significance to Biomedical Research and the Program of the Institute: The reported decline in the functional capacity of lymphocytes with age may be intrinsic and/or extrinsic. These possibilities can be tested by modifying components in an in vitro system which tests functional capacity and by assessing the effects of freeze-thaw damage on lymphocytic biomembrane systems. If the age-related differences in lymphocytic functional capacity are dependent on thymidine metabolism, this offers a potential for modifying cellular metabolism to provide an enhanced immune response in older individuals. Controlled rate cooling is a new technique that may be used to separate subpopulations of lymphocytes for further study at the cellular level.

Proposed Course: The characterization of possible age-related changes in mammalian lymphoid cells will include further studies on the enhancement of splenic lymphocytic responses to mitogens as these may be related to cellular biomembrane systems. Age-related lymphocytic resistance to stress will be tested using controlled rate freezing techniques.

Publications:

Brock, M.A.: Circannual rhythms. I. Free-running rhythms in growth and development of the marine cnidarian, Campanularia flexuosa. Comp. Biochem. Physiol. 51A: 377-383, 1975.

Brock, M.A.: Circannual rhythms. II. Temperature-compensated free-running rhythms in growth and development of the marine cnidarian, Campanularia flexuosa. Comp. Biochem. Physiol. 51A: 385-390, 1975.

Brock, M.A.: Circannual rhythms. III. Rhythmicity in the longevity of hydranths of the marine cnidarian, Campanularia flexuosa. Comp. Biochem. Physiol. 51A: 391-398, 1975.

Brock, M.A.: Free-running rhythmicity in the life spans of hydranths of the marine cnidarian, Campanularia flexuosa. J. Interdisciplinary Cycle Res., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-AG-00097-02-LCP

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

The Use of Lectins and Other Molecular Probes to Study Cell Surface Receptors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Joel H. Shaper Staff Fellow LCP NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Cellular and Comparative Physiology

SECTION

INSTITUTE AND LOCATION NIA, NIH, Gerontology Research Center, Baltimore City Hospitals, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.65	0.65	0.0

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this project are to (1) isolate, characterize and compare unique lymphocyte plasma membrane glycoprotein receptors which bind mitogenic and nonmitogenic lectins possessing the same monosaccharide binding specificity; and (2) chemically produce a monovalent wheat germ lectin by the introduction of a sugar analogue covalently into the carbohydrate binding site, thereby correlating intrinsic valency of a lectin with biological activity.

Objectives: The ability to identify and correlate changes at the cell surface or membrane level as a consequence of cellular aging or of a specific biological insult such as viral transformation requires, as a prerequisite, a precise knowledge of the membrane structure. It is the purpose of this project to develop procedures whereby specific glycoprotein receptors and enzymatic activities which are intrinsic components of the plasma membrane can be identified (visualized by transmission or scanning electron microscopy) and isolated for subsequent physicochemical characterization. The objectives of this project are to:

1. Visualize and determine the topographical distribution of the membrane bound Na^+-K^+ ATPase (Na^+-K^+ pump).

2. Isolate, characterize and compare unique lymphocyte plasma membrane glycoprotein receptors which bind mitogenic and nonmitogenic lectins possessing the same monosaccharide binding specificity.

3. Chemically produce a monovalent wheat germ lectin by the introduction of a sugar analogue covalently into the carbohydrate binding site, thereby correlating intrinsic valency of a lectin with biological activity.

Methods Employed: Five chemically active analogues of N-acetyl glucosamine have been synthesized by published procedures when available or by devised procedures.

All analogues have been chemically characterized and found to be highly reactive toward model compounds. The ability of each analogue to interact specifically with the carbohydrate binding site of WGA was monitored by standard hemagglutinin assay and by affinity chromatography on N acetyl-glucosamine-1-hexanol-6-amine-Sepharose 4B, an affinity resin highly specific for WGA. Stoichometrics of affinity labelling were determined on the amino acid analyzer following standard procedures.

Major Findings: Four of the five synthesized analogues, although highly reactive toward low molecular weight compounds, did not react covalently with any part of the WGA polypeptide chain or with the carbohydrate binding site. The data can be used to begin to map the binding cleft of WGA. The lack of affinity labelling with either the epoxide or the iodo analogues suggests the absence of a sulfhydryl, primary amine, histidine, tyrosine or carboxyl group in the region of the carbohydrate binding site which interacts with the 1 or 2 position of N-acetyl glucosamine. One analogue was found to react with WGA after exposure to light. Interaction of the analogue specifically with the carbohydrate binding site of WGA was indicated by the following: (1) WGA no longer bound to N-acetyl-glucosamine-Sepharose-4B affinity columns, (2) the titer of 1 mg/ml solution of WGA was reduced five-fold, and (3) the analogue was not dissociated from WGA or Gu·HCl. Quantitation of the amount of N-acetyl glucosamine bond per mole of WGA after denaturation with SDS and exhaustive dialysis indicated that 1.5-1.9 (Δ = 1.68)

moles of sugar per mole of WGA monomer. The exact location of the analogue has not been determined. However, the quantitative data indicates a relatively high amount of spurious attachment of N-acetyl glucosamine in a position other than the carbohydrate site. Clarification of this point is now in progress.

Significance to Biomedical Research and the Program of the Institute: Attention is focused on the thesis that alterations in cell membrane structure constituents are manifestations of changes in cellular regulatory mechanisms. Cell surface alterations are well documented in the case of viral transformation and conceivably may be diagnostic for events leading to the production of an "aging cell." The development of the methodologies to isolate and to study the localization and distribution of unique cell surface receptors and specific enzymatic activities can support or eliminate such a hypothesis.

Proposed Course: This project was terminated on 2/28/76.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-AG-00098-02-LCP

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Characterization of Alterations in Lymphocyte Membrane Structural Components

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. W. Heine	Staff Fellow	LCP NIA
OTHER:	W. H. Adler	Medical Officer, PHS	LCP NIA

COOPERATING UNITS (if any)

None

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Laboratory of Cellular and Comparative Physiology

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INSTITUTE AND LOCATION NIA, NIH, Gerontology Research Center, Baltimore City Hospitals, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.95	0.95	0.0

SUMMARY OF WORK (200 words or less - underline keywords)

The study on the characterization of alterations in membrane structural components of lymphocytes with senescence requires an understanding of lymphocytic responses in immunogenic interactions, which decrease with age. The project is concerned with the quantitative aspect of in vitro activated T lymphocytes. The assay involves the infection of activated lymphocytes with vesicular stomatitis virus (VSV) and the subsequent plating of infected cells on T cell monolayers for determination of virus plaque-forming cells (V-PFC). The effect of interferon, a byproduct of T cell activation on the V-PFC assay, is examined. In addition, the interferon, as well as the initial number of T lymphocytes responding to a stimulation, is compared between different mouse strains.

Objectives: Many lymphocytic responses in immunity are decreased with age. This decrease occurs with a normal number of T or B cells being present in the lymphoid organs and, therefore, must reflect a decrease in the number in a population which are functional or could reflect a decrease in the division potential of the population. This project is concerned with very early events in an in vitro lymphocyte response with a quantitative assay for triggered T and B cells.

Methods Employed: The assay makes use of the fact that lymphocytes which are resting are impervious to infection with virus, while a lymphocyte which is triggered by a mitogenic stimulus can be infected. Therefore, the assay is for the number of infected cells which is determined by using lytic virus and distributing the lymphoid cells on a monolayer and determining the number of plaques which appear. Since interferon can inhibit viral infection, it must be considered as a part of the assay. Interferon can be measured by determining the inhibition of viral RNA synthesis using a radiolabelled RNA precursor. Further studies are concerned with methods to determine the changes in a lymphocyte membrane which allows the cell to be infected. These methods are standard metabolic assays for the turnover of membrane glycoprotein and lipoprotein along with membrane associated enzyme assays for cyclic nucleotide synthesis.

Major Findings: The number of activated lymphocytes early in a mitogen-stimulated culture represents a very low percentage of the population of lymphocytes in culture. The total number of cells responding to a Con A stimulation is in the range of 0.001%. Activated cell numbers increase at a rapid rate over the first 24 hours of culture. The rate reflects probable cell division, recruitment, and possibly the delayed activation of other populations. However, the number of activated cells increases at a logarithmic rate for the first 24 hours of culture. There are less activated cells in the spleen from older animals, while the rate of increase remains the same as the young cells. The assay for activated cells becomes unusable after 48 hours of culture due to interferon production engendered by the mitogen stimulus. Interferon induction is dependent on the mitogen used and on the strain and age of the cell donors. Cells from older animals of certain strains produce a large quantity of interferon, even without a mitogenic stimulus. It is possible then that the cells from the older mice are carrying a virus and this may be a cause of the lesser number of functional cells. A possible mechanism of cellular damage by a non-lytic virus can be shown by the experiments in which anti-viral antibody in the presence of complement can lyse cells which are virus infected and which carry viral antigen on their membrane.

Significance to Biomedical Research and the Program of the Institute: These studies provide an insight into very early cellular events in an immune response. These events are not able to be studied any other way. Also, the role of interferon in host response and as an indicator of viral disease may help to diagnose sub-clinical pathology in aging mammals.

Proposed Course: To determine possible causes of interferon induction in aging mice and to determine methods of increasing the number of responsive cells in an immune reaction. Also to outline the role of virus and antibody to virus in the destruction of immunocompetent T cells.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-AG-00099-01-LCP

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Immune Status of Balb/c Mice during Induction of Myologenous Neoplasia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. Makinodan	Chief, LCCP	LCP NIA
OTHER:	S. Hausman	Staff Fellow	LCP NIA
	M. M. B. Kay	Medical Officer, PHS	LCP NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

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INSTITUTE AND LOCATION NIA, NIH, Gerontology Research Center, Baltimore City Hospitals, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.45	0.45	0.0

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to determine the nature of the correlation between immunodeficiency and neoplasia. The project was prematurely terminated 12 months after its initiation by a Sendai epizootic infection of the GRC/NIA animal colony which began in January, 1976.

Project Description:

Objectives: A correlation exists between immunodeficiency and "immunodeficient" diseases (i.e., neoplasia, autoimmunity, and infection). The purpose of this project is to determine the nature of the correlation (i.e., is the deficiency responsible for the disease?, is the disease responsible for the deficiency?, or are both a consequence of another event?).

Methods Employed: The effect of naturally occurring immunodeficiency states (such as aging) and induced immunodeficient states upon the induction of neoplasia in Balb/c mice will be assessed.

Major Findings: This project, which would have taken 2-3 years to complete, was terminated 12 months after its initiation by a Sendai epidemic in the GRC/NIA animal colony. Therefore, no major findings can be reported.

Significance to Biomedical Research and the Program of the Institute: The results of these studies are fundamental to our understanding of the relationship between age-related immunodeficiency and disease.

Proposed Course: Project terminated because of Sendai epizootic infection which began in January, 1976.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-AG-00100-01-LCP

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Regulation of Expression of Autoimmune Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. M. B. Kay	Medical Officer, PHS	LCP NIA
OTHER:	T. Makinodan	Chief, LCCP	LCP NIA
	S. Hausman	Staff Fellow	LCP NIA

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Cellular and Comparative Physiology

SECTION

INSTITUTE AND LOCATION NIA, NIH, Gerontology Research Center, Baltimore City Hospitals, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.75	0.55	1.2

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to determine the mechanism by which the expression of autoimmune disease is regulated using NZB and NZB hybrids. Unfortunately, this project was terminated nine months after its initiation by epizootic Sendai infection which entered the GRC/NIA animal colony in January, 1976.

PHS-6040
(1-75)

Project Description:

Objectives: Autoimmune disease is expressed in autoimmune disease-prone NZB mice and in hybrids of NZB and autoimmune resistant CBA mice. However, the disease is expressed a year later in the hybrids. This suggests that the presence of CBA genes has modified the expression of autoimmune disease. The purpose of this project is to determine the mechanism by which the expression of autoimmune disease is regulated using NZB hybrids as model systems.

Methods Employed: Autoimmune disease-prone NZB and their hybrids which have either delayed or accelerated expression of autoimmune disease will be studied, and the severity, manifestations, and time of onset of the disease will be assessed.

Major Findings: This project, which would have taken 3 years to complete, was terminated 9 months after initiation when a Sendai epidemic swept the GRC/NIA animal colony in January, 1976. Therefore, no major findings were obtained.

Significance to Biomedical Research and the Program of the Institute: The results of these studies are vital to our understanding of the regulation mechanism of age-associated immunodeficiency diseases.

Proposed Course: Project terminated because of epizootic Sendai infection.

Publications: None

The Laboratory of Molecular Aging conducts studies which provide the information needed to understand the fundamental mechanisms underlying the aging process. Our research this year has continued to emphasize two critical areas known to undergo perturbations that lead to the inability of organisms to maintain homeostasis, namely, physiological control and genetic information transfer systems. These investigations have impact on the mechanisms of age-dependent alterations in (1) renal function, (2) cardiac function, (3) skeletal muscle activity, and (4) metabolism, and (5) on mechanisms basic to the development of aging hypotheses which transcend specific physiological systems.

The thrust of the work on control systems is directed towards membrane biology. The problems and questions studied relate to: (a) molecular organization; (b) mechanisms of transmembrane vectorial transport; (c) membrane reception of hormonal stimuli, the transduction and propagation of these stimuli, and the regulation of membrane activity; (d) catalytic and integrative function; (e) turnover of membranes; and (f) failure to maintain membrane structure and function, leading to cell death.

An essential step in understanding the mechanisms by which heart muscle contractility changes with age is to define the transport processes which are basic to regulation of cardiac function. One such system being investigated is the transport and intracellular compartmentation of Ca^{2+} by the sarcoplasmic reticulum. The duration of the active state in cardiac muscle is determined in part by the duration of the interval in which Ca^{2+} is bound to myofibrillar regulatory proteins; consequently, the rate of removal of Ca^{2+} by sarcoplasmic reticulum will influence the time course of muscle relaxation. Preparations of sarcoplasmic reticulum from hearts of old (24-25 months) rats showed significantly less uptake than membranes from hearts of young (6-8 months) animals over a range of physiologically relevant Ca^{2+} concentrations. This age-dependent change in Ca^{2+} transport activity is consistent with the observation that hearts of aged animals show a prolonged relaxation phase and suggests a possible biochemical mechanism for that change.

The release of sequestered Ca^{2+} is crucial to the suggested role of the sarcoplasmic reticulum and/or mitochondria in controlling muscle contraction through regulation of cytosol Ca^{2+} concentration. The mechanism of this release is unknown. Depolarization of the membranes has been postulated. Sarcoplasmic reticulum membranes from rabbit skeletal muscle were polarized and the membrane vesicles accumulated Ca^{2+}, upon addition of ATP. The membranes were successfully depolarized by using salts with lipophilic anions, and this was shown by measuring fluorescence of the probe molecule, 1-anilino-8-naphthalenesulfonic acid (ANS). However, Ca^{2+} was not released. Only the Ca^{2+} ionophore A23187 was effective. Attempts to induce the release of Ca^{2+} accumulated by mitochondria (rat heart, liver, kidney or rabbit heart, liver) by potential physiological mechanisms were similarly unsuccessful. Addition of the ionophore or uncoupling agents effected release but cyclic AMP did not. Thus, the report of Borle on the action of cyclic AMP could not be confirmed. These studies indicate that the mechanisms of release of Ca^{2+} must be complex and, as yet, eludes definition.

Physiological concentrations of free Ca^{2+} inhibited isocitrate dehydrogenase, the key enzyme controlling Krebs cycle activity, thus, synthesis of ATP. Ca^{2+} acts as an allosteric inhibitor, decreasing the affinity of the enzyme for substrate. ADP is a positive effector of the enzyme, increasing the affinity for isocitrate. These findings plus the molecular orientation in the muscle of isocitrate dehydrogenase and other crucial enzymes that were reported previously to be sensitive to Ca^{2+} provide support for our hypothesis that muscle contraction, glycolytic flux and respiration would be maximal when the cytosolic concentration of Ca^{2+} is high and the reticulum and intramitochondrial concentrations are low. Conversely, relaxation of the muscle would be favored and metabolism would be inhibited when the cytosolic concentration of Ca^{2+} is low and the sequestered Ca^{2+} concentrations are high.

An additional opportunity to examine the impact of membrane biology on aging processes is found in the close relationship between the properties of renal membranes and kidney function. As noted previously, renal function itself is altered by age. Moreover, in other illnesses, such as congestive heart failure, renal adjustments to maintain fluid and solute homeostasis are slower in the aged. The plasma membrane of the proximal tubule epithelial cell has been found to be differentiated ultrastructurally, biochemically, and functionally into a luminal brush border membrane and a peritubular basal-lateral membrane. This year, techniques were developed to isolate the basal-lateral membrane. This preparation, together with an earlier procedure for isolating the brush border membrane, now enables us to examine in model membrane systems the mechanisms by which solutes vectorially enter and leave the tubular cell and how the functions of the membranes are coordinated by hormones to regulate transport processes.

Understanding the mechanism of the Na^+ gradient-dependent uptake of D-glucose by renal brush border membrane vesicles was advanced by studying the effect of the membrane potential on the transport process. Assuming that the Nernst relationship is applicable, membrane potentials were established by varying the K^+ concentration inside and outside the vesicle. Efflux of K^+, down its electrochemical gradient, was induced by the specific ionophore valinomycin and this was made concomitant with the electrogenic influx of Na^+ cotransported with D-glucose. The initial rate of D-glucose uptake was linearly related to log $[K^+i]/[K^+o]$, i.e. proportional to the membrane potential.

Considerable progress was made in defining the mechanisms of amino acid transport into renal brush border membrane vesicles. In addition to the dibasic amino acid transport system described last year, distinct Na^+ gradient-dependent, uphill (active), electrogenic transport systems were characterized for the neutral amino acids L-alanine and glycine, and the imino acid L-proline. In contrast, a Na^+ gradient-dependent, uphill, but electroneutral uptake system was identified for the acidic amino acid L-glutamic acid. All the above systems were stereospecific for the natural isomer and specific for the amino acid class. These findings provide strong support for the concept of multiple amino acid transport systems in kidney (and other tissues). These studies also provide the pioneering evidence to indicate that the specific aminoaciduria disorders observed in man may result from defects at the luminal membrane of the proximal tubule.

Significant advances were made in describing the mechanism of Na^+ uptake into renal brush border membrane vesicles. Na^+ was taken up into at least two spaces: one, which is not osmotically active and presumed to represent binding of Na^+ to the membrane surface; the other, which is osmotically active, and represents intravesicular Na^+ accumulation. Binding was found to be a saturable process, occuring at two or more sites: one, characterized by high affinity and low capacity; the other, by lower affinity but higher capacity. Binding is most compatible with polar-type interactions which suggests that the membranes carry fixed charges and in the presence of Na^+ would be expected to generate a Donnan potential. The major component of the transport of Na^+ into membrane vesicles was electroneutral. The ionophore nigericin stimulated Na^+ uptake in both K^+- and H^+-loaded vesicles, indicating that the membranes can maintain and utilize a chemical gradient for Na^+ transport in the presence of an electroneutral carrier. Moreover, an intravesicular/extravesicular H^+ gradient alone enhanced the initial rates of Na^+ uptake as well as supported an overshoot of the equilibrium. The transient nature of this accumulation of Na^+ against its concentration gradient suggests that the membrane itself possesses an electroneutral H^+-Na^+ carrier. The symmetry of this exchange was demonstrated by experiments in which the efflux was enhanced by an external proton gradient. In addition to Na^+-H^+ exchange, we found Na^+ counter-transport, i.e. Na^+-Na^+ exchange. K^+ enhanced Na^+ efflux, thus providing evidence for K^+-Na^+ exchange. These findings provide clues as to the mechanism of Na^+ transport which may be applicable to the fields of hypertension and other various salt retention or loss states.

Preparations of the basal-lateral segment of the plasma membrane of the renal tubule cell could best be evaluated biochemically by enrichment in the specific activity of ouabain-sensitive Na^+K^+ATPase. The isolated membranes were free of contamination by nuclei, lysosomes, and cytosol, but some mitochondrial and luminal membrane fragments were present.

Transport studies with basal-lateral membrane vesicles suggest that the mechanisms by which D-glucose and L-proline exit from the tubule is markedly different from the mechanisms by which these solutes enter the epithelial cell from the lumen. Data to date indicate that the transports across the basal-lateral membrane are Na^+-independent, suggesting non-facilitated downhill transport.

Investigations on the hormonal regulation of physiological control systems were focused on the metabolism and interactions of cyclic nucleotides, cAMP and cGMP, with membrane target sites. Renal brush border and basal-lateral membranes were used as models, for with these isolated preparations the sites of action can be pinpointed and the actions of the effectors may in time be correlated with specific functions. Adenylate cyclase, which mediates the synthesis of cAMP, was largely confined to the basal-lateral membrane. Parathyroid hormone and calcitonin markedly stimulated the basal level of enzyme activity, indicating the presence of receptors for the hormones on this membrane. Isoproterenol and epinephrine also enhanced activity, and this activation was completely blocked by propranolol. Prostaglandins, especially PGE and PGA, were potent stimulators. Vasopressin did not enhance activity, suggesting that the action of this hormone is restricted to the medullary region of the kidney. NaF and GMP-PNP, which presumably act at the catalytic and regulatory site of the enzyme, respectively, also increased activity.

The kidney possesses very high guanylate cyclase activity, resembling brain and lung in this respect. The enzyme was localized in the basal-lateral and brush border membranes and in the cytosol. The synthesis of cGMP in the basal-lateral membrane was found to be latent, full activity being elaborated by detergents and azide. Guanylate cyclase in the membrane was activated by ATP. The hormone secretin, which stimulates guanylate cyclase in the pancreas and liver, was without effect on the renal membrane enzyme but activated the soluble enzyme slightly. Carbamylcholine, which increases cGMP levels in nerve tissues, did not activate the renal enzyme.

Permeability properties of membranes are presumably regulated by phosphorylation/dephosphorylation of membrane proteins catalyzed by protein kinases and phosphatases. We previously found that renal brush border membranes phosphorylated endogenous proteins and added protamine and histones, phosphorylation of the latter being stimulated by cAMP. To determine whether there was one or multiple protein kinases, the membranes were extracted with detergent, with no loss in activity, and chromatographed on DE 52 columns. A symmetrical kinase I and an asymmetrical kinase IIa+b were resolved. Kinase I was specific for protamine and completely cAMP-independent. Kinase IIa+b showed activity with histone f_2b, lys-rich histone, protamine, casein, and phosvitin. cAMP-activation was found with only listone f_2b as substrate and was located in IIa. IIb activities with histone f_2b and other substrates were cAMP-independent. IIa activity was inhibited by cAMP-dependent kinase protein inhibitor (Walsh et al.). cAMP binding pattern coincided with IIa. IIa and b were resolved by chromatography in presence of cAMP. The values of Km for ATP for the different kinases differ. Ka = 1.3×10^{-8} and 9.4×10^{-7} for cAMP and cGMP, respectively; V was the same. These findings indicate that this segment of the renal tubular cell plasma membrane contains at least three protein kinases. Comparison of the protein kinases in the brush border segment of the plasma membrane with that in the basal-lateral region suggests that the activities in the two membranes are not identical.

Significant advances were made in assessing the nature of controls of cyclic nucleotide levels in cells via regulation of cyclic nucleotide phosphodiesterase activity. Multiple forms of the brush border membrane cAMP phosphodiesterase activity, dependent on concentration of substrate, were found. When assayed with 1 μM or 1 mM cAMP, activities differed in pH optimum, effects of various divalent cations, inhibition by metal ion chelators and reactivation by metals, thermolability, sensitivity to inhibitors, and specificity. Renal brush border membranes also possessed cGMP phosphodiesterase activity which was distinct from the cAMP phosphodiesterase. Enzymes which hydrolyze cyclic nucleotides were also found in the basal-lateral membrane and in the cytosol. The role of the soluble enzymes in controlling levels of cyclic nucleotides is most intriguing because of the co-presence of an activator protein whose action is dependent on Ca^{2+}. Rapid-quench techniques were applied to this study and the preliminary findings suggest control of cGMP hydrolysis by Ca^{2+} in kidney, heart, and brain, in a time frame of 0-300 msec. The activity in brain is particularly sensitive to this regulation, and this takes on added importance since it has been proposed that the actions of cholinergic agents are mediated via cGMP.

Last year, we reported that genetic strains of *Drosophila* presumably having "null" mutations at NAD-linked α-glycerophosphate dehydrogenase locus, as measured *in vitro*, had decreased longevity, severely restricted flight ability, and premature deterioration and atrophy in flight muscle ultrastructure, seen previously only in aged flies, but that one stock adapted and regained the ability to fly despite the continued absence of measurable enzyme activity. The mechanism of this compensation was the subject of continued investigation. Measurement of steady-state concentrations of metabolic intermediates and their responses to mechanical agitation of the flies revealed that both mutant and compensated strains lack an effective NAD-α-glycerophosphate dehydrogenase *in vivo*. Thus, with physical activity there was a pronounced rise in the ratios dihydroxyacetonephosphate/α-glycerophosphate and dihydroxyacetonephosphate/3-phosphoglycerate. The latter suggests that the adapted mutants have not evolved an effective mechanism for oxidizing NADH produced during glycolysis. Consistent with this view was the low steady-state concentration of pyruvate in all mutant strains. Normal citrate concentrations, despite the low pyruvate levels, were found. This raised the possibility of fatty acid or amino acid oxidation. However, experiments with isolated mitochondria ruled this out, and failed to show any differences in mitochondrial metabolism between strains. Thus, the mechanism of the compensation remains obscure.

The effect of age of the blowfly on the pattern of response of intermediary metabolites to the energy demand of flight was investigated. It became clear that the nutritional status of the senescent fly was of paramount importance. There were no differences in the concentrations of any of the metabolites measured between carbohydrate + protein-fed young and senescent flies. When senescent carbohydrate-fed flies were compared with young carbohydrate-fed flies there were pronounced differences, however. The concentrations of all of the glycolytic and tricarboxylate intermediates measured were depressed in the senescent, sugar-fed flies. This does not reflect simply a smaller total pool of intermediates due to protein deprivation, however, as the decrease in some intermediates was much more marked than in others. Thus, whereas dihydroxyacetonephosphate and α-glycerophosphate concentrations were 77 and 67% of the values in young flies (on 30 sec flight), 3-phosphoglycerate, phosphoenolpyruvate and pyruvate concentrations were only 42, 41 and 24%, respectively. This pattern suggests a rather selective loss of activity of glyceraldehyde-3-phosphate dehydrogenase and pyruvate kinase. Although the concentrations of citrate and malate were much reduced in the senescent, sugar-fed flies, this need not reflect an alteration in activities of tricarboxylate cycle enzymes, as the low concentration of pyruvate which follows from the lesion in glycolysis would be expected to lead to reduced levels of all tricarboxylate cycle intermediates. The enzymes glyceraldehyde-3-phosphate dehydrogenase and pyruvate kinase were therefore assayed in flight muscle extracts from young adult and senescent flies, under both dietary conditions. It was found that glyceraldehyde-3-phosphate dehydrogenase activity was diminished in the senescent, carbohydrate-fed flies, in keeping with the conclusions from the intermediate study discussed above. However, there was no detectable decrement in activity of pyruvate kinase, nor of trehalase, hexokinase or phosphofructokinase, all enzymes catalyzing irreversible, and therefore potentially rate-limiting, reactions in glycolysis. In conclusion, it is felt that the approach of studying the response of key intermediates

to the change in flux through a metabolic pathway has considerable application to aging research. The reason is that it directs attention to the changed activities of enzymes (or conceivably transport processes) which actually exert an effect in vivo on the flux through a pathway, and it is the flux through the pathway which is primarily of significance to the animal. These enzymes can then be studied in vitro. Such an approach avoids time being wasted in the study of small decrements with age in the activity of enzymes which catalyze reactions very close to equilibrium. On the other hand, should such a decrement be large enough to affect the overall flux through the pathway, then it will be reflected in the intermediate pattern. This allows a rational approach and avoids the necessity for screening every one of the several hundred enzymes involved in intermediary metabolism.

The research program on the propagation of genetic information focuses on the interactions of DNA and RNA with each other, with proteins, and with metal ions. These are studied with the object of understanding the interactions in terms of biological function, particularly in the replication of DNA, the transcription of RNA, and protein synthesis. A primary objective is to determine under what conditions metal ions are essential for information transfer, and under what conditions they produce errors in the information.

Aging human fibroblast cells in tissue culture contained increasing amounts of Ca^{2+}, K^{+}, Mg^{2+}, Fe^{2+}, and Zn^{2+}.

Metal ions have profound effects upon the structure of nucleoproteins, and it was previously shown that age changes in nucleoprotein structure are correlated with changes induced by metal ions. Clues to an understanding of such changes came from a study of the effect of metal ions on the interaction between DNA and polylysine, which mimics the lysine - phosphate bonding of the DNA - histone complexes in chromatin. In the absence of divalent metal ions DNA-polylysine associated in an ordered anisotropic (handed) pattern characterized by a high negative ellipticity in the CD spectrum, which we call $\psi(-)$. Metal ions binding to DNA phosphate (e.g. Mg^{2+}) enhanced the $\psi(-)$ effect, but certain metal ions that bind to nucleotide bases (e.g. Cu^{2+}) produced a CD spectrum with high positive ellipticity, which we call $\psi(+)$, and which indicates that the directional twist of the associated molecules was reversed. Some histones formed $\psi(+)$ complexes with DNA and others formed $\psi(-)$ complexes. Metal binding can reversibly convert one form into the other.

Metal ions produced a variety of deleterious changes in the structure of nucleic acids and nucleotides. Copper(II) ions disordered the helical structure of polynucleotides by forming both intramolecular crosslinks within and between the polymer strands. Lead ions dephosphorylated nucleotides to nucleosides to a degree that depends on the position of the phosphate and the presence of a hydroxyl group adjacent to the phosphate. Most metal ions that can depolymerize polynucleotides cannot dephosphorylate the nucleotide end products of the depolymerization.

Macromolecules, man-made or modified by man, are an important part of the human environment; for these reasons the biological effects of representative water soluble macromolecules were studied. The results show that foreign, non-degradable macromolecules were partly taken up by human cells grown in

culture and stayed associated with them for many generations. In animals foreign non-degradable macromolecules were rapidly distributed in the body and only a small portion of the injected macromolecules was excreted; in organs the foreign macromolecules were found even a month after application. This situation must be compared with the finding that potential beneficiary effects on animals of a non-degradable polymer [poly-9-vinyladenine protection against viral leukemia] were of only about one day's duration. On the other hand, only negligible beneficial effects in protection against viruses were obtained with macromolecules which were degraded in less than an hour. Thus, the search for potential polymeric drugs should concentrate on compounds of intermediate stability.

Metal ions that are present in the erythrocyte can have important effects on the oxidation and oxygen affinity of hemoglobin. Comparative studies on hemoglobins from various species have been carried out to determine the nature of the binding sites of these ions. These studies indicate that Zn(II), which increases the oxygen affinity of hemoglobin, binds to a site that includes cysteine β-93 and histidine β-143. Similar studies have demonstrated that human and rabbit hemoglobins possess a high affinity Cu(II) binding site involving histidine β-2 which is not present in sheep, bovine, and horse hemoglobins. The hemoglobins from all these species contain a copper binding site of lower affinity that is responsible for its oxidation. The high affinity site in human and rabbit hemoglobin, therefore, protects the hemoglobin from oxidation by low Cu(II) concentrations.

Investigations on human blood samples from participants of the longitudinal program support a hypothesis relating age changes in the erythrocyte to tissue hypoxia. Increases in 2,3-DPG in older individuals suggest oxygen deprivation. Increases in total glutathione suggest a possible breakdown in the usual mechanisms for maintaining reduced functional hemoglobin. Studies on a limited number of individuals support such a breakdown; in older individuals there was about 3% more oxidized nonfunctional hemoglobin and the *in vitro* rate of autoxidation was about 50% higher. The level of oxidized hemoglobin was still quite low and would probably not produce hypoxia. However, the presence of low concentrations of oxidized hemoglobin, the need for additional glutathione to reduce hemoglobin, as well as the higher Zn(II) concentration found in older individuals, all tend to increase the oxygen affinity of hemoglobin. Such an increase can limit the ability to release oxygen to the tissues, which could produce tissue hypoxia.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00041-03 LMA

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Physiological Control Systems and Aging I
Membrane Transport Mechanisms

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)

None

LAB/BRANCH

Gerontology Research Center, Laboratory of Molecular Aging

SECTION

Intermediary Metabolism Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.9	4.9	2.0

SUMMARY OF WORK (200 words or less - underline keywords)

This study of membrane transport is targeted to provide the basic scientific information needed to understand the mechanisms whereby age-dependent perturbations in physiological control systems lead to the inability of the organism to maintain homeostasis. Membrane vesicles derived from the luminal brush border segment and the peritubular basal-lateral region of the renal tubule epithelial cell plasma membrane are used as model systems. Topics investigated include: 1) the role of the membrane potential in the Na^+ gradient-dependent uptake of D-glucose by brush border membrane vesicles; 2) the mechanisms and specificities of amino acid transport systems; 3) the uptake systems for Na^+, Na^+-H^+, Na^+-Na^+, and Na^+-K^+ exchanges; 4) the development of methods for the isolation and the characterization of the basal-lateral region of the renal tubule plasma membrane; 5) the comparison of the mechanism by which solutes enter and exit the tubular epithelial cell.

PHS-6040
(1-75)

Project Description:

Objectives: These studies are targeted to provide the information needed to understand the mechanisms whereby age-dependent perturbations in physiological control systems lead to the inability of the organism to maintain homeostasis. The research has impact on the mechanism underlying age-related changes in renal function. The thrust of the work is focused on questions dealing with the biology of membranes, including: (1) molecular organization; (2) role in selective vectorial transport; (3) hormonal regulation of function; (4) catalytic function: (5) turnover; and (6) failure to maintain structure, leading to cell death.

Methods Employed: Membrane vesicles derived from the luminal brush border segment and the peritubular basal-lateral region of the renal tubule epithelial cell plasma membrane are used as model systems.

Major Findings: Last year, we reported that the uptake of D-glucose into renal brush border membrane vesicles was Na^+ gradient-dependent, stereospecific, uphill, and electrogenic and suggested that in the intact kidney the asymmetric distribution of Na^+ across the proximal tubular cell and the electrochemical potential across the luminal membrane provide the driving force to transport D-glucose against its concentration gradient. Understanding the mechanism of the system was advanced by studying the effect of the membrane potential on the transport process. Assuming that the Nernst relationship is applicable, membrane potentials were established by varying the K^+ concentration inside and outside the vesicle. Efflux of K^+, down its electrochemical gradient, was induced by the specific ionophore valinomycin and this was made concomitant with the electrogenic influx of Na^+ cotransported with D-glucose. The initial rate of D-glucose uptake was linearly related to log $[K^+i]/[K^+o]$, i.e. proportional to the membrane potential.

Comparison of the initial rates of the Na^+-stimulated D-glucose transport into K^+ loaded vesicles, in the presence and absence of valinomycin, and in which Na^+ and K^+ salts of specific anions were used showed that the permeability of the anion contributed to the electrochemical diffusion potential. Thus, these findings with model membrane systems support the Goldman (1942) theoretical modification of the Nernst equation as it relates to transport systems.

Amiloride, which was reported to inhibit Na^+ transport across frog skin and toad bladder (Bentley, 1968), did not affect Na^+-dependent D-glucose uptake in brush border membrane vesicles. Although amiloride inhibited the unidirectional movement of Na^+ across the proximal tubule in the intact rat, the inhibition was explained by a decrease in the permeability to Na^+ in the intercellular, rather than the intracellular pathway (Wilczewski et al., 1974). This may also explain why amiloride did not affect the active transport potential in the rat proximal tubule (Fromter and Gessner, 1975). Two diuretics, furoseamide and ethacrynic acid, did not interfere with the Na^+-dependent D-glucose uptake in isolated vesicles. This is consistent with the observation that furoseamide acts primarily on active Cl^- transport in the ascending loop of Henle (Burg and Green, 1973).

Considerable progress was made in defining the mechanisms of amino acid transport into renal brush border membrane vesicles. In addition to the dibasic amino acid transport system described last year, distinct Na^+ gradient-dependent, uphill (active), electrogenic transport systems were characterized for the neutral amino acids L-alanine and glycine, and the imino acid L-proline. In contrast, a Na^+ gradient-dependent, uphill, but electroneutral uptake system was identified for the acidic amino acid L-glutamic acid. All the above systems were stereospecific for the natural isomer and specific for the amino acid class. These findings provide strong support for the concept of multiple amino acid transport systems in kidney (and other tissues). These studies also provide the pioneering evidence to indicate that the specific aminoaciduria disorders observed in man may result from defects at the luminal membrane of the proximal tubule.

Significant advances were made in describing the mechanism of Na^+ uptake into renal brush border membrane vesicles. Na^+ was taken up into at least two spaces: one, which is not osmotically active and presumed to represent binding of Na^+ to the membrane surface; the other, which is osmotically active, and represents intravesicular Na^+ accumulation. Binding was found to be a saturable process, occuring at two or more sites: one, characterized by high affinity and low capacity; the other, by lower affinity but higher capacity. An alternative explanation for the observed heterogeneity is that Na^+ exerts a negative cooperative effect on its binding. An estimate of the high affinity binding constant was made at 0° and 20° and from this an estimate of the standard enthalpy of binding. From these estimates we conclude that binding is most compatible with polar-type interactions which suggests that the membranes carry fixed charges and in the presence of Na^+ would be expected to generate a Donnon potential. This was supported by experiments with arginine, a positively charged amino acid, which is exlcuded in part from the intravesicular space at equilibrium by the presence of Na^+.

The major component of the transport of Na^+ into membrane vesicles is electroneutral. It was not affected by manipulations which are electrogenic. For example, 100 μM Na^+ uptake was independent of incubation of K^+-loaded vesicles with valinomycin or H^+-loaded vesicles with FCCP. On the other hand, the ionophore nigericin stimulated Na^+ uptake in both K^+- and H^+-loaded vesicles, indicating that the membranes can maintain and utilize a chemical gradient for Na^+ transport in the presence of an electroneutral carrier. Moreover, an intravesicular/extravesicular H^+ gradient alone enhanced the initial rates of Na^+ uptake as well as supported an overshoot of the equilibrium. The transient nature of this accumulation of Na^+ against its concentration gradient suggests that the membrane itself possesses an electroneutral H^+-Na^+ carrier. The symmetry of this exchange was demonstrated by experiments in which the efflux was enhanced by an external proton gradient. In addition to Na^+-H^+ exchange, we found Na^+ countertransport, i.e. Na^+-Na^+ exchange. K^+ enhanced Na^+ efflux, thus providing evidence for K^+-Na^+ exchange. These findings provide clues as to the mechanism of Na^+ transport which may be applicable to the fields of hypertension and other various salt retention or loss states.

Preparations of the basal-lateral segment of the plasma membrane of the renal tubule cell could best be evaluated biochemically by enrichment in the specific activity of ouabain-sensitive Na^+K^+ ATPase. Relative to that in the homogenate of the cortex, the specific activity of the enzyme in the membrane preparation was increased 7-fold. The isolated membranes were free of contamination by nuclei, lysosomes, and cytosol, but some mitochondrial and luminal membrane fragments were present. The purity of the preparation was examined additionally by electron microscopy. Only traces of contamination by other cell components were observed. The electron micrographs also showed that the membranes, when isolated, vesiculated. This indicates that the basal lateral membranes can be utilized for studies of the mechanisms by whch solutes exit form the tubular cell.

Techniques were also developed for the preparation of both the basal-lateral and brush border membranes from the same kidney. This achievement is of significance because it enables examination of the two membranes from the same animal, which is of particular importance in aging studies in which aged animals are not plentiful. To be noted also, is that the procedure developed for the isolation of membranes from the rabbit were successfully utilized for preparing membranes from the dog. Studies on the aged dog were initiated.

Transport studies with basal-lateral membrane vesicles suggest that the mechanisms by which D-glucose and L-proline exit from the tubule is markedly different from the mechanisms by which these solutes enter the epithelial cell from the lumen. Data to date indicate that the transports across the basal-lateral membrane are Na^+-independent, suggesting non-facilitated downhill transport.

Significance to Biomedical Research and to the Program of the Institute: These studies, using renal plasma membrane vesicles as model membranes to investigate transport processes, provide the information needed to understand the mechanisms whereby age-dependent perturbation in physiological control systems lead to the inability of the aged organism to maintain homeostasis.

Proposed Course of the Project: Studies on the mechanisms of transport will be continued to further probe the relationship between membrane potential and uphill solute translocation, define the nature of the coupling of Na^+ flux to the movements of cotransported solutes, and characterize the transport systems in the basal-lateral membrane. New studies will be initiated to make use of the recent techniques of resolution and reconstitution to examine the function of membrane transport systems in terms of molecular organization. The development of techniques and meaningful information on membrane biology will be applied to the study of membrane transport systems in the kidney of the aged dog.

Publications:

Aronson, P. and Sacktor, B.: The Na^+ gradient dependent transport of D-glucose in renal brush border membranes. J. Biol. Chem. 250: 6032-6039, 1975.

Beck, J. and Sacktor, B.: The energetics of the Na^+-dependent transport of D-glucose in renal brush border membrane vesicles. J. Biol. Chem. 250: 8674-8680, 1975.

Liang, C. T. and Sacktor, B.: Bicarbonate-stimulated ATPase in the renal proximal tubule luminal (brush border) membrane. Arch. Biochem. Biophys., 1976, in press.

Sacktor, B.: The Brush Border of the Proximal Renal Tubule and the Intestinal Mucosa. In Jamieson, G. A., and Robinson, D. M. (Eds.): Mammalian Cell Membranes. London, Butterworths, 1976, Vol. 4, in press.

Sacktor, B.: Transport in Membrane Vesicles Isolated from the Mammalian Kidney and Intestine. In Sanadi, R. (Ed.): Current Topics in Bioenergetics. New York, Academic Press, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00042-03 LMA

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Physiological Control Systems and Aging II
Mechanisms of Hormonal Regulation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	R. Balakir	Chemist	LMA NIA
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COOPERATING UNITS (if any)
None

LAB/BRANCH
Gerontology Research Center, Laboratory of Molecular Aging

SECTION
Intermediary Metabolism Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.8	2.8	2.0

SUMMARY OF WORK (200 words or less - underline keywords)

This study of hormonal regulation of membrane function is targeted to provide the information needed to understand the mechanisms whereby age-dependent perturbations in physiological control systems lead to the inability of the organism to maintain homeostasis. Investigations are focused on the biochemical interactions of hormones, mediated via cyclic AMP and cyclic GMP, with membrane systems. Topics investigated include: 1) hormone receptors in membranes; 2) adenylate cyclase and guanylate cyclase activities; 3) phosphorylation of membrane proteins by cyclic nucleotide-dependent and -independent protein kinases; 4) control of cyclic nucleotide levels by regulation of phosphodiesterase activities.

Project Description:

Objectives: This study of hormonal regulation of membrane function is targeted to provide the information needed to understand the mechanisms whereby age-dependent perturbations in physiological control systems lead to the inability of the organism to maintain homeostasis. Investigations are focused on the biochemical interactions of hormones, mediated via cyclic AMP and cyclic GMP, with membrane systems. The research has impact on the mechanism underlying age-related changes in renal, cardiac, and neural function.

Methods Employed: Membranes and cytosol preparations derived from the renal plasma membrane, heart and brain are used as model systems.

Major Findings: Investigations on the hormonal regulation of physiological control systems were focused on the metabolism and interactions of cyclic nucleotides, cAMP and cGMP, with membrane target sites. Renal brush border and basal-lateral membranes were used as models, for with these isolated preparations the sites of action can be pinpointed and the actions of the effectors may in time be correlated with specific functions. Adenylate cyclase, which mediates the synthesis of cAMP, was largely confined to the basal-lateral membrane. Parathyroid hormone and calcitonin markedly stimulated the basal level of enzyme activity, indicating the presence of receptors for the hormones on this membrane. Isoproterenol and epinephrine also enhanced activity, and this activation was completely blocked by propranolol. Prostaglandins, especially PGE and PGA, were potent stimulators. Vasopressin did not enhance activity, suggesting that the action of this hormone is restricted to the medullary region of the kidney. NaF and GMP-PNP, which presumably act at the catalytic and regulatory site of the enzyme, respectively, also increased activity. Combinations of hormones or GMP-PNP resulted in stimulations that were additive.

Brush border membrane preparations contained relatively low adenylate cyclase activity which could not be fully accounted for by contamination with basal-lateral membranes, as judged from the Na^+K^+ATPase activities found in the two membranes. Adenylate cyclase activity in the brush border membrane was also sensitive to hormones. No evidence for latent enzyme activity was observed as detergents (Triton X-100) markedly decreased activity in both membranes.

The kidney possesses very high guanylate cyclase activity, resembling brain and lung in this respect. The enzyme was localized in the basal-lateral and brush border membranes and in the cytosol, and these activities could be readily distinguished. The synthesis of cGMP in the basal-lateral membrane was found to be latent, full activity being elaborated by detergents and azide. Guanylate cyclase in the membrane was activated by ATP. The hormone secretin, which stimulates guanylate cyclase in the pancreas and liver, was without effect on the renal membrane enzyme but activated the soluble enzyme slightly. Carbamylcholine, which increases cGMP levels in nerve tissues, did not activate the renal enzyme. Neither parathyroid hormone, calcitonin, nor insulin affected guanylate cyclase.

Permeability properties of membranes are presumably regulated by phosphorylation/dephosphorylation of membrane proteins catalyzed by protein kinases and phosphatases. We reported last year that renal brush border membranes phosphorylated endogenous proteins and added protamine and histones, phosphorylation of the latter being stimulated by cAMP. To determine whether there was one or multiple protein kinases, the membranes were extracted with detergent, with no loss in activity, and chromatographed on DE 52 columns. A symmetrical kinase I and an asymmetrical kinase IIa+b were resolved. Kinase I was specific for protamine and completely cAMP-independent. Kinase IIa+b showed activity with histone f_2b, lys-rich histone, protamine, casein, and phosvitin. cAMP-activation was found with only histone f_2b as substrate and was located in IIa. IIb activities with histone f_2b and other substrates were cAMP-independent. IIa activity was inhibited by cAMP-dependent kinase protein inhibitor (Walsh et al.). cAMP binding pattern coincided with IIa. IIa and b were resolved by chromatography in presence of cAMP. The values of Km for ATP for the different kinases differ. Ka = 1.3×10^{-8} and 9.4×10^{-7} for cAMP and cGMP, respectively; V was the same. These findings indicate that this segment of the renal tubular cell plasma membrane contains at least three protein kinases. Comparison of the protein kinases in the brush border segment of the plasma membrane with that in the basal-lateral region suggests that the activities in the two membranes are not identical.

Significant advances were made in assessing the nature of controls of cyclic nucleotide levels in cells via regulation of cyclic nucleotide phosphodiesterase activity. Multiple forms of the brush border membrane cAMP phosphodiesterase activity, dependent on concentration of substrate, were found. When assayed with 1 μM or 1 mM cAMP, activities differed in pH optimum, effects of various divalent cations, inhibition by metal ion chelators and reactivation by metals, thermolability, sensitivity to inhibitors, and specificity. Renal brush border membranes also possessed cGMP phosphodiesterase activity which was distinct from the cAMP phosphodiesterase. Enzymes which hydrolyze cyclic nucleotides were also found in the basal-lateral membrane and in the cytosol. The role of the soluble enzymes in controlling levels of cyclic nucleotides is most intriguing because of the co-presence of an activator protein whose action is dependent on Ca^{2+}. Rapid-quench techniques were applied to this study and the preliminary findings suggest control of cGMP hydrolysis by Ca^{2+} in kidney, heart, and brain, in a time frame of 0-300 msec. The activity in brain is particularly sensitive to this regulation, and this takes on added importance since it has been proposed that the actions of cholinergic agents are mediated via cGMP.

Significance to Biomedical Research and to the Program of the Institute: These studies provide information needed to understand the mechanisms whereby age dependent alterations in the hormonal regulation of physiological control systems lead to the inability of the aged organism to maintain homeostasis.

Proposed Course of the Project: Studies will continue on the mechanisms by which hormones, whose actions are mediated via cAMP and cGMP, regulate physiological activities in the kidney, heart, and brain. Emphasis will be given to defining how phosphorylation/dephosphorylation of membrane proteins

affect transport across renal plasma membranes and cardiac sarcoplasmic reticulum. The regulation of cyclic nucleotide hydrolysis by Ca^{2+} in heart and brain will be pursued by a transient kinetic approach. New studies will be initiated to resolve, reconstitute and restore competence to membranes showing age-related alterations in hormonal receptors and cyclic nucleotide metabolism. Radioimmunoassays for both cAMP and cGMP will be established to measure levels of cyclic nucleotides in tissues and cells in various physiological and growth states. These will include rat ventricular muscles to assess age changes in catecholamine responsiveness and early and late passage human fibroblasts. Studies on cyclic nucleotide metabolism and the action of hormones on membrane systems will be extended to the kidney of the aged dog and human cells in culture.

Publications:

Filburn, C. and Sacktor, B.: Cyclic nucleotide phosphodiesterases of rabbit renal cortex. I. Characterization of brush border membrane activities. Arch. Biochem. Biophys., 174: 249-261, 1976.

DHEW/PHS/NIH/OD/DRG (Rev. 10-74) PROJECT NUMBER (Do NOT use this space)

	INTRAMURAL RESEARCH PROJECT	Z01 AG 00043-03 LMA

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Physiological Control Systems and Aging III
Regulation of Intermediary Metabolism

NAMES, LABORATORY AND INSTITUTE AFFILIATION, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	R. Hamsford	Visiting Scientist	LMA NIA
	R. Johnson	Visiting Fellow	LMA NIA

COOPERATING UNITS (if any)

NONE

LAB/BRANCH
Gerontology Research Center, Laboratory of Molecular Aging

SECTION
Intermediary Metabolism Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.3	3.3	3.0

SUMMARY OF WORK (200 words or less - underline keywords)

This study of the regulation of intermediary metabolism is targeted to provide the information needed to understand the mechanisms whereby age-dependent perturbations in physiological control systems lead to the inability of the organism to maintain homeostasis. Topics investigated include: 1) the effect of aging on energy metabolism; 2) the role of Ca^{2+} in the control of skeletal and cardiac muscle activity; 3) the mechanism of Ca^{2+} release from sarcoplasmic reticulum and mitochondria; 4) the interactions of carbohydrate and lipid metabolism; 5) the mechanism of oxidative phosphorylation.

Project Description:

Objectives: These studies are targeted to provide the basic information needed to understand the mechanisms whereby age-dependent perturbations in physiological control systems lead to the inability of the organism to maintain homeostasis. The thrust of the work is focused on questions dealing with membrane biology including (1) molecular organization; (2) mechanisms of transmembrane vectorial transport; (3) catalytic and integrative function; (4) turnover of membranes; (5) failure to maintain membrane structure and function, lead to cell death. The research has impact on the mechanism underlying age-related changes in skeletal muscle activity, cardiac function and metabolism.

Methods Employed: As model systems, mitochondria isolated from insect flight muscle and mammalian cardiac muscle are employed. Also used are rabbit skeletal muscle sarcoplasmic reticulum membranes.

Major Findings: Last year, we reported that genetic strains of Drosophila presumably having "null" mutations at NAD-linked α-glycerophosphate dehydrogenase locus, as measured in vitro, had decreased longevity, severely restricted flight ability, and premature deterioration and atrophy in flight muscle ultrastructure, seen previously only in aged flies, but that one stock adapted and regained the ability to fly despite the continued absence of measurable enzyme activity. The mechanism of this compensation was the subject of continued investigation. Measurement of steady-state concentrations of metabolic intermediates and their responses to mechanical agitation of the flies revealed that both mutant and compensated strains lack an effective NAD-α-glycerophosphate dehydrogenase in vivo. Thus, with physical activity there was a pronounced rise in the ratios dihydroxyacetonephosphate/α-glycerophosphate and dihydroxyacetonephosphate/3-phosphoglycerate. The latter suggests that the adapted mutants have not evolved an effective mechanism for oxidizing NADH produced during glycolysis. Consistent with this view was the low steady-state concentration of pyruvate in all mutant strains. Normal citrate concentrations, despite the low pyruvate levels, were found. This raised the possibility of fatty acid or amino acid oxidation. However, experiments with isolated mitochondria ruled this out, and failed to show any differences in mitochondrial metabolism between strains. Thus, the mechanism of the compensation remains obscure.

The effect of age of the blowfly on the pattern of response of intermediary metabolites to the energy demand of flight was investigated. It became clear that the nutritional status of the senescent fly was of paramount importance. There were no differences in the concentrations of any of the metabolites measured between carbohydrate + protein-fed young and senescent flies. When senescent carbohydrate-fed flies were compared with young carbohydrate-fed flies there were pronounced differences, however. The concentrations of all of the glycolytic and tricarboxylate intermediates measured were depressed in the senescent, sugar-fed flies. This does not reflect simply a smaller total pool of intermediates due to protein deprivation, however, as the

decrease in some intermediates was much more marked than in others. Thus, whereas dihydroxyacetonephosphate and α-glycerophosphate concentrations were 77 and 67% of the values in young flies (ou 30 sec flight), 3-phosphoglycerate, phosphoenolpyruvate and pyruvate concentrations were only 62, 41 and 24%, respectively. This pattern suggests a rather selective loss of activity of glyceraldehyde-3-phosphate dehydrogenase and pyruvate kinase. Although the concentrations of citrate and malate were much reduced in the senescent, sugar-fed flies, this need not reflect an alteration in activities of tricarboxylate cycle enzymes, as the low concentration of pyruvate which follows from the lesion in glycolysis would be expected to lead to reduced levels of all tricarboxylate cycle intermediates. The enzymes glyceraldehyde-3-phosphate dehydrogenase and pyruvate kinase were therefore assayed in flight muscle extracts from young adult and senescent flies, under both dietary conditions. It was found that glyceraldehyde-3-phosphate dehydrogenase activity was diminished in the senescent, carbohydrate-fed flies, in keeping with the conclusions from the intermediate study discussed above. However, there was no detectable decrement in activity of pyruvate kinase, nor of trehalase, hexokinase or phosphofructokinase, all enzymes catalyzing irreversible, and therefore potentially rate-limiting, reactions in glycolysis. In conclusion, it is felt that the approach of studying the response of key intermediates to the change in flux through a metabolic pathway has considerable application to aging research. The reason is that it directs attention to the changed activities of enzymes (or conceivably transport processes) which actually exert an effect in vivo on the flux through a pathway, and it is the flux through the pathway which is primarily of significance to the animal. These enzymes can then be studied in vitro. Such an approach avoids time being wasted in the study of small decrements with age in the activity of enzymes which catalyze reactions very close to equilibrium. On the other hand, should such a decrement be large enough to affect the overall flux through the pathway, then it will be reflected in the intermediate pattern. This allows a rational approach and avoids the necessity for screening every one of the several hundred enzymes involved in intermediary metabolism.

Physiological concentrations of free Ca^{2+} inhibited isocitrate dehydrogenase, the key enzyme controlling Krebs cycle activity, thus, synthesis of ATP. Ca^{2+} acts as an allosteric inhibitor, decreasing the affinity of the enzyme for substrate. This finding plus the molecular orientation in the muscle of isocitrate dehydrogenase and other crucial enzymes that were reported previously to be sensitive to Ca^{2+} provide support for our hypothesis that muscle contraction, glycolytic flux and respiration would be maximal when the cytosolic concentration of Ca^{2+} is high and the reticulum and intramitochondrial concentrations are low. Conversely, relaxation of the muscle would be favored and metabolism would be inhibited when the cytosolic concentration of Ca^{2+} is low and the sequestered Ca^{2+} concentrations are high.

Because, as reported last year, the NAD-linked isocitrate dehydrogenase has been identified as the pacemaker of the tricarboxylate cycle in flight msucle, the kinetic parameters of this complex enzyme were studied in detail. In addition to Ca^{2+}, ADP, inorganic phosphate, citrate, pH, Mn^{2+} or Mg^{2+}, were found to be effectors of the enzyme and they interacted with each other and

with substrates isocitrate and NAD. To illustrate with one example, ADP was shown to be an activator of the enzyme, increasing the affinity of the dehydrogenase for isocitrate. However, the extent of activation by ADP was dependent upon the concentration of isocitrate. Thus at low concentrations of isocitrate (below 1 mM), 3 mM ADP caused a 60-80 fold increase in dehydrogenase activity. At 2 mM isocitrate, the activation was only 19-25 fold while in the presence of 3-4 mM isocitrate, the activation was 2-5 fold. Thus, the apparent Ka of ADP in the activation of the enzyme is a function of the isocitrate concentration, decreasing with increasing concentrations of isocitrate. At 0.057 mM isocitrate the K_{ADP} was found to be 1.8 mM, at 0.34 mM isocitrate it was 1.2 mM, and at 2 mM isocitrate it was 0.7 mM.

In other studies on the control of oxidations and phosphorylation in blowfly flight muscle mitochondrial membranes, redox potential differences were measured during controlled and active oxidation coincident with measurements of phosphate potential ([ATP]/[ADP]+[Pi]) and the gradient in the electrochemical activity of protons across the mitochondrial membrane (the protonmotive force). Protonmotive force acts as the intermediate between mitochondria electron transfer and the phosphorylation of ADP, according to the Mitchell chemiosmotic hypothesis on the mechanism of oxidative phosphorylation. It was found that on activating respiration, the redox potential increased but the protonmotive force decreased, a finding apparently inconsistent with the theory.

Studies on the control of cardiac metabolism was directed to determine the mechanism whereby lipids inhibit carbohydrate oxidation in rat heart. In model experiments it was found that high ATP/ADP, acetyl-CoA/CoASH and NADH/NAD ratios favored the phosphorylation, and inactivation, of pyruvate dehydrogenase, mediated by pyruvate dehydrogenase kinase. These ratios presumably would be increased by lipid oxidation.

The release of sequestered Ca^{2+} is crucial to the suggested role of the sarcoplasmic reticulum and/or mitochondria in controlling muscle contraction through regulation of cytosol Ca^{2+} concentration. The mechanism of this release is unknown. Depolarization of the membranes has been postulated. Sarcoplasmic reticulum membranes from rabbit skeletal muscle were polarized and the membrane vesicles accumulated Ca^{2+}, upon addition of ATP. The membranes were successfully depolarized by using salts with lipophilic anions, and this was shown by measuring fluorescence of the probe molecule, 1-anilino-8-naphthalenesulfonic acid (ANS). However, Ca^{2+} was not released. Only the Ca^{2+} ionophore A23187 was effective. Attempts to induce the release of Ca^{2+} accumulated by mitochondria (rat heart, liver, kidney or rabbit heart, liver) by potential physiological mechanisms were similarly unsuccessful. Addition of the ionophore or uncoupling agents effected release but cyclic AMP did not. Thus, the report of Borle on the action of cyclic AMP could not be confirmed. These studies indicate that the mechanisms of release of Ca^{2+} must be complex and, as yet, eludes definition.

Significance to Biomedical Research and to the Program of the Institute:
These studies provide the basic scientific information needed to understand the mechanisms by which the ability of tissues, e.g. skeletal muscle, heart, to perform maximally declines with age.

Proposed Course of the Project: The effect of age on the metabolic control in skeletal muscle will be continued using insect flight muscle as a model system. The interaction between carbohydrate and lipid metabolism in mammalian heart will be pursued and a comparison will be made for possible differences in this interaction between hearts of young and aged rats. A longer term project will be the application of the study of steady-state concentrations of intermediates, as described for the fly, to the perfused rat heart. The response to an energy demand can be investigated by altering the pressure against which the heart is working, freeze-clamping, and determining the concentrations of key metabolites. This would permit a direct comparison of the way the pathways of catabolism adjust to increased energy demand in hearts from young and old animals.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00044-03 LMA (including Z01 AG 00045-02 LMA)

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Effects of Metals and Proteins on Nucleic Acids, Information Transfer, and Aging.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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7.3	5.3	2.0

SUMMARY OF WORK (200 words or less - underline keywords)

This project focuses on the interaction of molecules concerned with genetic information transfer. A primary objective is to determine under what conditions metal ions are essential for information transfer, and under what conditions they produce errors in the information and may thus contribute to biological aging. Topics of interest are: (1) The effects of metal ions on the structure of nucleoproteins and chromatin; (2) Age related changes in chromatin structure; (3) Interactions of ribosomes; (4) Dephosphorylation of nucleotides by metal ions; (5) Crosslinking of nucleic acid strands by metal ions; (6) The effects of metal ions on RNA polymerase; (7) Metal ions and cellular aging.

Project Description:

Objectives: (1) To determine specificities in the binding of proteins to nucleic acids that are responsible for biological activity, (2) to elucidate the structure of chromatin, (3) to understand how aging may be related to nucleoprotein structure, (4) to study the structure of the ribosome and its interaction with other substances engaged in protein synthesis, (5) to understand the participation of metal ions in the biological activities of nucleic acids and nucleoproteins, (6) to obtain clues to the reasons for the toxicity of metal ions, (7) to understand the role of metal ions in the aging process, (8) to use metal ions as probes to study the mechanism of biological information transfer.

Methods Employed: (1) The interaction of metal ions, proteins and nucleic acids are studied by optical rotatory dispersion, circular dichroism, and spectrophotometry to determine conformational changes, and by infrared, nuclear magnetic resonance, and electron spin resonance techniques. (2) Chromatin structure is analyzed by means of model systems to determine which characteristics of chromatin are responsible for its function. (3) The interaction of ribosomes, messenger RNA, transfer RNA and other factors involved in protein synthesis are studied by optical rotatory dispersion, circular dichroism, and tracer analysis. (4) Effects of metal ions are studied to determine under what conditions they serve an essential function in information transfer, and under what conditions they induce errors in the information content. (5) Metal ion effects are studied in tissue culture to determine intracellular biological activity.

Major Findings: A. Reversible change in ψ structure of DNA-Polylysine complexes induced by Metal Binding. Metal ions have profound effects upon the structure of nucleoproteins, and it has been previously shown that age changes in nucleoprotein structure are correlated with changes induced by metal ions. These changes are believed to be due to changes in the way in which DNA and proteins are packed together in chromatin. We have used the DNA - polylysine system as a model for this DNA - protein interaction since much of this interaction is due to binding of lysine to DNA phosphate. The binding sites on DNA determine the effect of metal ions on nucleic acid structure; they are also instrumental in determining the effects on nucleoprotein structure. Metal ions binding to DNA phosphate (Mg^{2+}, Mn^{2+}, Co^{2+}, Zn^{2+}, etc.) enhance the ψ effect, a highly ordered anisotropic (handed) packing of the DNA - polylysine complex, characterized by high negative ellipticity of the CD spectrum. On the other hand, certain metal ions binding to the DNA bases promote a quite different type of packing characterized by high positive ellipticity, which we have designated the $\psi(+)$ state. We call the more common packing the $\psi(-)$ state. That the $\psi(+)$ state is produced by metal binding to bases is clear from two experiments. At room temperature Cu(II) produces an enhanced $\psi(-)$ effect, but heating causes a transition to the $\psi(+)$ state; we have previously established that heating increases base binding of Cu(II), which can bind to both phosphate and bases. The complex cis$[Pt(NH_3)_2Cl_2]$, which reacts slowly with DNA to form Pt bonds to the bases, produces the $\psi(+)$ state at room temperature, and the ellipticity increases gradually with time. Cu(II) can be used for a reversible transition between $\psi(-)$ and $\psi(+)$; removal of

Cu(II) from the ψ(+) complex by EDTA or high salt causes reversion to ψ(-). The significance of this phenomenon is apparent when one considers that some histones binding to DNA produce the ψ(+) state, while others produce the ψ(-) state. We have also found that synthetic double helical polynucleotides can be made to form either ψ(+) or ψ(-) complexes with polylysine. Both poly dGC and poly dAT form ψ(+) complexes by direct mixing with polylysine and ψ(-) complexes by gradient dialysis. Melting studies indicate that DNA is double helical in both ψ(+) and ψ(-) states, and we believe that the ψ(+) state arises from the packing of DNA in the A form while the ψ(-) state is an aggregate of DNA in the B form. If that is so, base binding metal ions convert B to A and therefore cause the transition from ψ(-) to ψ(+). DNA in a packed state in the chromosome is known to undergo a conformational change from B to A during transcription. Some base binding metals do not produce the ψ(+) state; we do not presently know why not.

B. Age related changes in chromatin structure. Most of the possible age changes that could occur in the structure of chromatin, e.g. in DNA or protein structure or the binding of protein to DNA, should lead to a change in the accessibility of DNA to chemical or enzymatic action. A study of the age change in DNA accessibility is therefore a probe of changes in the structure of chromatin. We have previously reported that no significant age changes could be detected in soluble chromatin isolated from human fibroblast cells in tissue culture (WI-38 cells). We considered that chromatin solubilization may destroy structural components that change with age. We therefore selected a procedure for the isolation of chromatin that minimizes such structural changes but retains the chromatin in a partly insoluble condition. The chromatin was prepared in this way from old (24-26 months) and young (8-9 months) rat livers from the GRC colony. It was pretreated with RNase and then reacted with micrococcal nuclease, which is an effective probe of DNA accessibility. The reaction was followed both by u.v. absorbance and the more specific diphenylamine reaction. In spite of the heterogeneity of the reaction mixture very reproducible kinetic experiments could be obtained. The results from the diphenylamine reaction indicate that DNA does in fact become more accessible with age, as indicated by increasing hydrolysis with age. The u.v. results lead to the same conclusion, but more u.v. absorbing material is produced than one would expect from the diphenylamine results. We do not know at present what this extra material is but assume that old nuclei are stickier than young nuclei to cytoplasmic contaminants. On the other hand, the young chromatin appears to contain more RNA than old chromatin. This finding comes from studies in which the chromatin was not pretreated with ribonuclease; under these conditions more total nucleic acid is hydrolyzed in young than in old chromatin. The major findings were obtained from a comparison of chromatin from four young and three old rats. No overlap existed between the values for young and old, but a larger number of experiments are required before these age changes are unequivocally established.

C. Metal ions in young and old cells. We have previously reported a generalized increase in the concentration of metal ions in late passage of WI-38 cells as compared to early passage cells. These results were obtained with frozen cell packs obtained from Dr. Leonard Hayflick, and were attributed

to strongly bound metal. These results can now be compared with results obtained with live cells. The same generalized increase in concentration of metal ions per cell is observed for Ca^{2+}, K^{+}, Mg^{2+}, Fe^{2+}, and Zn^{2+} in live cells. The absolute concentrations of metal ions present in large quantities, i.e. Ca^{2+}, K^{+}, and Mg^{2+}, are much higher in the live cells than in the frozen cells, indicating leakage from the frozen cells. The concentration of trace metal ions such as Fe^{2+} and Zn^{2+} are not very different in live and frozen cells. Our analytical technique (atomic absorption spectrophotometry) yields highly reproducible results when applied to the same samples, and we have checked a Bureau of Standards analyzed sample of beef liver. However, the results with cells are very dependent on the treatment of the cells, and old vs. young comparisons are valid only for identically treated cells.

D. Ribosomal conformational changes and RNase II binding. We have previously reported that the binding of poly(U), $tRNA^{phe}$ and phe-$tRNA^{phe}$ to e. coli. ribosomes results in a variety of changes in the circular dichroism (CD) of the ribosome. These changes were interpreted as changes in the ribosomal conformation during the binding processes that accompany protein synthesis. We then discovered, however, that the poly(U) was degraded upon association with the ribosome. Further investigation proved that the observed CD changes were similar to the CD differences between intact and degraded poly(U). It was therefore concluded that a nuclease activity was associated with our ribosomal preparation. Such activity was also found in ribosomes given to us by H. Weissbach and by M. Cashel. Ribosomes prepared here and by H. Weissbach were then subjected to sedimentation on a sucrose gradient. The nuclease activity was separated from the ribosomes in this way. The nuclease degraded poly(U) to yield 5'UMP as exclusive end product and proceeded without accumulation of long chain intermediates. This mode of action is characteristic of RNase II. The ribosomes from which this nuclease had been removed exhibits little or no change in CD when bound to poly(U) and tRNA. We conclude that, contrary to our previous report, messenger and transfer RNA binding to ribosome is not accompanied by ribosomal conformational changes that can be detected by CD measurements. We also conclude that ribosomes prepared and purified in the accepted manner in prestigious laboratories contain bound RNase II and that this fact is not generally realized by those who work with ribosomes.

E. Dephosphorylation of Nucleotides by lead. In line with our interest in studying the deleterious effects of metal ions on the nucleic acids we have previously studied the degradation of RNA by metal ions, particularly by zinc(II), for which the mechanism and specificity of degradation was carefully worked out. The end product of degradation by zinc is mononucleotide. It has been previously shown that some metal ions, e.g. Pb^{2+}, can go further than Zn^{2+}, and dephosphorylate the nucleotide to nucleoside. We have now examined the dephosphorylation reaction with the object of elucidating the differences in degradative behavior of metal ions such as Zn^{2+} and Pb^{2+}. Rates of dephosphorylation have been studied as a function of pH and Pb^{2+} concentration for isomers of AMP and dAMP. All of these nucleotides are partly dephosphorylated in the absence of metal ion at acid pH (optimum pH 4). Pb^{2+} ions actually inhibit acid dephosphorylation, but catalyze a much greater degree of dephosphorylation (up to 100%) at higher pH. The extent of

dephosphorylation depends on the relative positions of the phosphate and the OH groups. Thus 5'AMP and 5'dAMP react to a lesser extent than 3'AMP and 2'AMP. The latter have OH groups adjacent to phosphate groups. Possibly chelation of Pb(II) to OH and phosphate is involved, but other explanations are possible. Curiously, 3'dAMP, which is most extensively dephosphorylated by acid, exhibits little or no dephosphorylation by Pb^{2+}. A study of the CD spectra of the Pb(II) complexes of these nucleotides indicates that the metal ions stack the different isomers in quite different ways. A comparison of these CD spectra with those of the Zn(II) complexes reveals that Pb and Zn produce quite different stacking interactions. We believe that the tendency of the metal ions to dephosphorylate nucleotides, and the degree of this tendency, depends on the exact structure of the stacked nucleotides produced by a given metal with a given nucleotide. The stoichiometry of the lead nucleotide complexes is 3:2, as determined by precipitation and continuous variation studies, in contrast to 1:1 for the zinc nucleotide complexes.

F. Mechanism of disordering of polynucleotides by copper(II). Intra- molecular and intermolecular crosslinks. We have previously shown that Cu(II) induces a cooperative disordering of helical polynucleotides, and that this disordering involves the intermolecular crosslinking of poly- nucleotide strands by copper. Cooperativity constants have been calculated both for the disordering produced by the copper and for the binding of the copper itself to the polynucleotide. These constants indicate the degree to which each binding and disordering step enhances succeeding steps. It is found that the disordering is more cooperative than the binding; therefore each bound copper disorders more than one nucleotide, and it can be calculated that it disorders 4-5 nucleotides. We have then compared the cooperative disordering of poly(A) with hexa(A) and found that the polymer disordering requires much less Cu(II) and is more cooperative than the disordering of the hexamer. But since the cooperative unit was shown to be only 4-5 nucleotides in length, intermolecular crosslinking alone should lead to the same behavior for polymer and hexamer. The difference in behavior proves the presence of the intramolecular links in the polymer that are also indicated by other evidence; such links are sterically prevented in the hexamer. We have thus shown rather conclusively that the cooperative disordering of polynucleotides by copper involves both intermolecular and intramolecular crosslinks.

G. Effect of metal ions on RNA polymerase. We have previously shown that metal binding can (in the case of Pt complexes) decrease the size of the RNA message produced by *E. coli* RNA polymerases. We are now engaged in a study of the mechanism of activation of this enzyme by metal ions, one of which (Mg^{2+}) has been shown to incorporate only the correct ribonucleotides, while another (Mn^{2+}) has been shown to produce the error incorporation of deoxynucleotides. We have obtained a purified preparation of the holoenzyme suitable for physical chemical measurements. Determinations of enzyme activity as a function of metal concentration as well as kinetic studies reveal that the order of activating ability is $Mn^{2+}>Mg^{2+}>Co^{2+}$. Cu^{2+} and Gd^{3+} did not activate at all; the latter was tried because it is a good probe in NMR measurements. High concentrations of Mn^{2+} and Co^{2+}, but not of Mg^{2+}, are somewhat inhibitory.

Significance to Biomedical Research and to the Program of the Institute: The studies on the age changes in chromatin and metal contents of cells are of obvious relevance. The participation of metal ions in every aspect of genetic information transfer and the deleterious effects on this transfer, caused by undesired metal ions or essential metal ions in undesired concentrations make the study of metal ion interactions with nucleic acids of major importance. Metal ions are presumably not responsible for the primary events that cause aging, but we believe that they may be important factors in determining individual differences in the aging process. An understanding of the structure and function of chromatin (and therefore protein - DNA interaction), ribosomes, the nucleic acid polymerases, etc., is essential to an understanding of the aging phenomenon. We are particularly interested in studies that show how information transfer can go wrong.

Proposed Course of Project: Studies will be carried out to confirm the age changes that have been observed in chromatin and to correlate them and to understand them. We shall attempt to define more precisely the structural changes induced in DNA - polylysine through metal ion binding. We intend to carry out studies on the metal content of cells obtained from individuals of different ages. We expect to determine whether the efficiency of ribosomes in preventing errors in protein synthesis may change with age. Investigations on RNA polymerase will be continued with the object of determining how different metal ions produce more or less error in the incorporation of dexoynucleotide into RNA. Studies on the dephosphorylation and disordering of nucleic acids and their components by metal ions, and other effects of metal binding to nucleic acids, will be continued.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00046-06 LMA

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Polymers as Biological Reagents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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SECTION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

SUMMARY OF WORK (200 words or less - underline keywords)

Macromolecules, man-made or modified by man, are an important part of the human environment; for these reasons the biological effects of representative water soluble macromolecules have been studied. The results show that foreign, non-degradable macromolecules are partly taken up by human cells grown in glass and stay associated with them for many generations. In animals foreign non-degradable macromolecules are rapidly distributed in the body and only a small portion of the injected macromolecules can be excreted; in organs the foreign macromolecules are found even a month after application. This situation must be compared with the finding that potential beneficiary effects on animals of a non-degradable polymer [poly-9-vinyladenine protection against viral leukemia] are of only about one day's duration. On the other hand, only negligible beneficiary effects in protection against viruses were obtained with macromolecules which are degraded in less than an hour. Thus the search for potential polymeric drugs should concentrate on compounds of intermediate stability.

PHS-6040
(1-75)

Project Description:

Objectives: The project focuses on the effects of synthetic polymers on human cells grown in tissue culture and on the interaction of polymers with viral systems and age-related changes in these phenomena. The ultimate objective is to develop synthetic polymers which can be used as drugs.

Methods Employed: The study requires a rather broad range of chemical and biological methods. Most chemical syntheses and the in vitro work with viral enzymatic systems are performed at the Gerontology Research Center. The work with cellular enzymatic systems is done in collaboration with Dr. S. H. Wilson from the National Cancer Institute. The study of the replication of murine leukemia virus is in collaboration with Dr. P. Pitha, and the study of effects of polymers on animals is in collaboration with Dr. V. Vengris, both of the Department of Medicine, Johns Hopkins University.

Major Findings: A. Vinyl Analogs of Nucleic Acids. An antiviral polymer, poly-9-vinyladenine has very low toxicity to mammalian cells grown in culture or to animals. The inhibition of synthesis of viral nucleic acid is implicated as a cause of antiviral action, and to investigate the mechanistic reasons for the differential effects on cells and virus we studied the effects of poly-9-vinyladenine on viral and cellular DNA polymerases. It was found that poly(dT) directed poly(dA) synthesis by representatives of all three classes of cellular DNA polymerases are completely inhibited by the polymer; the use of different templates revealed that the enzyme activity was inhibited only in cases where base-pairing between the vinyl polymer and the template occurred. Similar results were obtained using polyribonucleotide templates and viral RNA dependent DNA polymerase. Thus the selective toxicity of poly-9-vinyladenine is not based on selective inhibitor - polymerase interaction and is probably based on the intracellular location of the drug or the differences in sequences of cellular and viral nucleic acids.

Intracellular localization and the fate of poly-9-vinyladenine in cells grown in culture was investigated using cells of strains of both mouse and human origin. It was found that only a small portion of the polymer is taken up by the cells, but this portion then remains associated with the cells for many generations.

The fate and the antiviral action of this polymer in animals was also investigated. In mice, after i.p. application the polymer slowly accumulates in liver, spleen and thymus and remains there for as long as a month. Thus this polymer, which suppresses the replication of murine leukemia virus, also accumulates in undegraded form in organs where the virus replicates. However, when the persistency of the antiviral effects of poly-9-vinyladenine in mice was investigated, it was found that daily doses of polymer were necessary for the sustained antiviral effects. Apparently the polymer is gradually segregated, in organs of the mouse, into cells or cell compartments away from primary sites of virus replication.

B. Peptidic Analogs of Nucleic Acids. The results on the fate and antiviral activity of poly-9-vinyladenine in mice suggest that for the polymeric drugs half lives in excess of 24 hours are without any significant advantage. On the other hand results which were obtained in collaborative work with Dr. R. T. Walker on the polyester analogs of nucleic acids indicate that polymers with half lives shorter than one hour do not have much pharmacological potency. To obtain polymeric analogs with intermediate half-lives, and presumably with optimal pharmacological effects, work on compounds with a peptidic backbone was initiated. As the starting point of the project the synthesis of amino acids carrying as substituents the bases of nucleic acids was attempted. At present four such amino acids have been prepared and their polymerization is presently being investigated.

Significance to Bio-medical Research and the Program of the Institute: Synthetic compounds of high molecular weight differ profoundly from low molecular weight compounds in their biological potentials. Macromolecules are distributed in a different way in animal tissues and are taken up by a different mechanism into cells, and also are degraded in a different way. The present studies aim to elucidate how to take advantage of these differences. Potential beneficiary effects may probably be obtained using macromolecules; on the other hand it is necessary to elucidate also the potential dangers which the soluble macromolecules may represent. The observed persistence of the intracellular polymers in cells demonstrates amply that accelerated deterioration of cells may result from the uptake of foreign macromolecules by cells from their environment.

Proposed Course of the Project: The present results show that synthetic polymers can elicit a selective biological effect similar to that of low molecular weight pharmaceuticals. By directed synthesis and study of the basic biological effects of polymers it is hoped to gain the knowledge necessary for the design of more potent preparations.

Publications:

Pitha, J., Stork, E. and Wimmer, E.: Protein synthesis during aging of human cells in culture. Exper. Cell Res. 94: 310-314, 1975.

Pitha, J.: Introduciton of N-vinyl group into tautomeric heterocycles by the exchange reaction. J. Org. Chem. 40: 3296-3298, 1975.

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Pitha, J. and Wilson, S. H.: Template specific inhibitor of mammalian DNA polymerases. Nucleic Acids Res. 3: 825-834, 1976.

Pitha, J. and Smid, J.: Ionophorous polymers: Interaction with polynucleotides and effects on RNA-directed DNA polymerase activity. Biochim. Biophys. Acta 425: 287-295, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00047-06 LMA

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Relation of Structure and Function in Hemoglobin

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. M. Rifkind	Research Chemist	LMA NIA
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	R. Lumry	Professor of Chemistry	Univ. of Minnesota
	M. Keyes	Research Chemist	Univ. of Minnesota

COOPERATING UNITS (if any)
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University of Minnesota, Minneapolis, Minnesota

LAB/BRANCH
Gerontology Research Center, Laboratory of Molecular Aging

SECTION
Molecular Chemistry

INSTITUTE AND LOCATION
NIH, NIA, Baltimore City Hospitals, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	0.7	0.5

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study the mechanisms involved in regulating the binding of oxygen to hemoglobin and the transport of oxygen to the tissues. The project also focuses on ways in which these functions are impaired and change with age. The following results can be reported: (1) zinc which increases the oxygen affinity of hemoglobin, binds to a site that includes cysteine β-93 and histidine β-143. (2) Human and rabbit hemoglobins possess a high affinity copper binding site involving histidine β-2 which is not present in sheep, bovine, and horse hemoglobin. The hemoglobins from all these species contain a copper binding site of lower affinity that is responsible for its oxidation. The high affinity site in human and rabbit hemoglobin therefore protects the hemoglobin from oxidation by low copper concentrations. (3) A comparison of blood samples from old and young humans show age increases in 2,3 DPG, gluthathione, oxidized nonfunctional hemoglobin, in vitro autoxidation rate, and zinc concentration. These changes support a hypothesis that age changes in the erythrocyte are related to tissue hypoxia. (4) Zinc increases the oxygen affinity of myoglobin, and calcium and magnesium decrease it. Calcium may have a role in regulating the release of oxygen in muscle.

PHS-6040
(1-75)

Project Description:

Objectives: (1) To study the binding of ligands to hemoglobin, and the role of the protein in controlling this function. (2) To study the mechanisms for maintaining hemoglobin in its functional form. (3) To study the mechanisms involved in regulating the transport of oxygen to the tissues.

Methods Employed: Various preparative procedures are used to purify hemoglobin and to separate various components of the erythrocyte. Visible, uv and atomic absorption spectroscopy, as well as gel electrophoresis is used to analyze for various erythrocyte components. The oxidation of hemoglobin is investigated under various conditions with and without the addition of various substances. Binding of metal ions and other small substances are studied by equilibrium dialysis. Effects on the oxidation and oxygenation are correlated with the binding of various substances.

Major Findings: A. Oxidation of Hemoglobin by Copper. We have previously found that Cu(II) binds to horse hemoglobin with a stoichiometry of one Cu(II) to every two hemes and rapidly oxidizes the β-subunit of hemoglobin. We find that human hemoglobin has a higher Cu(II) affinity and binds twice as much Cu(II) relative to horse hemoglobin. Nevertheless, higher Cu(II) concentration is necessary to rapidly oxidize human hemoglobin. These results indicate that human hemoglobin has a high affinity Cu(II) binding site from which Cu(II) is unable to oxidize the hemes. This binding, therefore, serves to protect human hemoglobin from oxidation by low concentrations of Cu(II) and may play a role in maximizing the oxygen transport capabilities of human erythrocytes.

These studies have been extended to include human A, A_2, and F hemoglobins as well as rabbit, sheep and bovine hemoglobins. The results indicate that all the human hemoglobins and rabbit hemoglobin have the high affinity Cu(II) binding site, while sheep, bovine and horse lack the high affinity Cu(II) binding site and are more readily oxidized. A comparison of the amino-acid sequence of these hemoglobins suggests that the high affinity Cu(II) binding site is in the region of the β-2 histidine residue.

B. The location of a zinc binding site which regulates the oxygenation of hemoglobin. There are appreciable concentrations of Zn(II) in the erythrocyte While much of this Zn(II) is associated with carbonic anhydrase, it is possible that some of the zinc may be available to interact with hemoglobin.

We have previously found that Zn(II) binds strongly to hemoglobin, producing a three-fold increase in the oxygen affinity. A comparison of the binding of Zn(II) to human hemoglobin A and human hemoglobin F with and without the β-93 sulfhydryls blocked, indicates a probable binding site for the Zn(II).

The cysteine residue at position 93 of the β-chain is adjacent to the histidine linked to the heme. We find that blocking this residue reduces the affinity of both hemoglobin A and F for zinc. Furthermore, hemoglobin F has a lower affinity for zinc than hemoglobin A whether or not the sulfhydryls are blocked. A comparison of the amino-acid sequence of hemoglobin A and F indicates that

the zinc binding site involves both the cysteine residue at position β-93 and the nearby histidine residue at position β-143.

C. Erythrocyte changes with age. We have previously proposed a hypothesis relating tissue hypoxia to possible erythrocyte changes with age, which affect the ability of the cell to regulate the oxygen affinity of hemoglobin. The validity of this hypothesis is being tested on human blood samples from participants of the longitudinal program. We find reasonably significant increases in 2,3-DPG in older individuals, which is suggestive of oxygen deprivation. We also find a significant increase in total glutathione, suggestive of a possible breakdown in the usual mechanisms for maintaining reduced functional hemoglobin. In support of such a breakdown, studies on a limited number of individuals suggest that in older individuals there is about 3% more oxidized nonfunctional hemoglobin and the in vitro rate of autoxidation is about 50% higher. The level of oxidized hemoglobin is still quite low and would probably not produce hypoxia. However, the presence of low concentrations of oxidized hemoglobin, the need for additional glutathione to reduce hemoglobin, as well as the higher Zn(II) concentration, which we find in older individuals, all tend to increase the oxygen affinity of hemoglobin. Such an increase can limit the ability to release oxygen to the tissues, which could produce tissue hypoxia.

D. The regulation of oxygen utilization in the muscle. While hemoglobin transports oxygen from the lungs to the tissues, the oxygen utilized in the muscles is stored by myoglobin. This protein has a structure very similar to the individual hemoglobin subunits. It has previously been reported that a particular small ionic substance in the muscle increases the oxygen affinity of myoglobin. Zn(II) has been found to further increase the oxygen affinity independently of the other ionic substance. However, Ca(II) and Mg(II) decrease the oxygen affinity of myoglobin by binding to this small ionic substance and releasing it from the myoglobin. Ca^{2+} ions are intimately involved in muscle contraction with the concentration of this ion in various compartments of the muscle undergoing large regulated changes. Therefore, the effect of Ca^{2+} on the oxygen affinity of myoglobin suggests a physiological role for Ca^{2+} in regulating the required release of oxygen within the muscle.

Significance to Bio-medical Research and to the Program of the Institute: The physiological role of hemoglobin is to transport oxygen from the lungs to the cells. The efficient uptake and release of oxygen requires cooperative oxygen binding and the proper regulation of the oxygen affinity. It is also necessary to limit the oxidation of hemoglobin in order to maintain an adequate concentration of functional hemoglobin. These studies thus help to elucidate a vital function of organisms. The aging process can involve changes in the ability of the organism to transport oxygen to certain tissues.

Proposed Course of the Project: (1) We plan to continue our studies in the various factors which influence the oxygenation and oxidation of hemoglobin in the erythrocyte and in purified systems. (2) We plan to continue our

studies on the age related changes in the level of various erythrocyte components. We also plan to determine whether there are age related changes in the amount of functional hemoglobin, and in the oxygenation characteristics within the erythrocyte. (3) Red cell fragility studies are planned to relate any observed changes in the erythrocyte of older individuals to possible changes in the age distribution or the rate of aging of the erythrocyte. (4) Studies are planned to determine the relationship between the binding of Cu(II) to hemoglobin and the oxidation of hemoglobin. Attempts will be made to determine where the Cu(II) responsible for oxidation binds and how electrons are transferred between Cu(II) and Fe(II).

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00048-02 LMA

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

The Mechanism of Metal Ion Transport Across Membranes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	N. W. Shock	Scientific Director	GRC NIA
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	R. L. Berger	Research Chemist	LTD NHLI
	R. W. Albers	Acting Chief, LNC	LNC NINDCDS
	R. Gobel	Research Chemist	LTB NCI
	M. Berman	Acting Chief, LTB	LTB NCI
	T. Ueda	Postdoctoral Res. Fellow	Yale Univ.
	P. Greengard	Prof., Dept. Pharmacology	Yale Univ.

COOPERATING UNITS (if any)

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Dept. Pharmacology, Yale Univ., New Haven, Ct.

LAB/BRANCH

Gerontology Research Center, Laboratory of Molecular Aging

SECTION

Molecular Chemistry

INSTITUTE AND LOCATION

NIH, NIA, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

SUMMARY OF WORK (200 words or less - underline keywords)

Systems involved in the active transport of metal ions subserve a diverse group of physiologic activities. One such transport system is the Ca^{2+} pump in sarcoplasmic reticulum which plays an essential role in the regulation of molecular events underlying muscular contraction and relaxation. The duration of the active state in cardiac muscle is determined in part by the duration of the interval in which Ca^{2+} is bound to myofibrillar regulatory proteins; consequently, the rate of removal of Ca^{2+} by sarcoplasmic reticulum will influence the time course of muscle relaxation. Sarcoplasmic microsomes prepared from old rat hearts showed significantly less uptake than microsomes from young hearts over a range of physiologically relevant Ca^{2+} concentrations. Estimates of the amount of protein in the preparation arising from other membranes revealed no significant age-related differences indicating that the difference in transport activity is not simply the result of a variable level of contamination. The age-dependent change in Ca^{2+} transport activity is consistent with the observation that old rat hearts show a prolonged relaxation phase and suggests a possible biochemical mechanism for that change.

PHS-6040
(1-75)

Project Description:

Objectives: (1) To understand the relation between calcium ion accumulation and the energy-linked partial reactions of ATP hydrolysis in sarcoplasmic reticulum (2) to determine the effects of age on the transient and steady state transport activity of cardiac microsomes (3) to measure the transient state kinetic behavior of enzymes involved in c-AMP dependent mechanisms in heart and brain (4) to characterize the enzymatic steps coupled to the transport of Na^+ and K^+ in animal cell membranes.

Methods Employed: The transient state kinetic effects of metal ion activators on adenosine triphosphatase from sarcoplasmic reticulum and electric organ membranes are being investigated by rapid chemical quenching. A chemical-stop multiple-mixing apparatus with the capability of resolving reaction half-lives of 5 milliseconds or larger has been developed for this purpose. A filtration unit which attaches to the quenching apparatus is used to characterize the initial phase of interaction of ^{45}Ca with sarcoplasmic reticulum vesicles prepared from rabbit skeletal muscle. The filtration apparatus consists of an aluminum block with a filtration port placed at right angles to the flow path. Reactants pumped through an off-set "T" mixer enter the flow path after passing through a short section of capillary tubing. The reaction time is varied by changing the flow rate and the volume of the capillary tubing between the points of mixing and filtration.

Major Findings: A. Ca^{2+} binding to sarcoplasmic reticulum microsomes: Rapid filtration experiments conducted at saturating levels of Ca^{2+} (10^{-5}M) show that steady state accumulation is preceeded by an initial burst or rapid phase of Ca^{2+} uptake. The time course of the binding reaction is slightly ahead of the Ca^{2+}-dependent phosphorylation reaction measured by transient state quenching experiments. The magnitude of the burst evaluated from the intercept of the y-axis and extrapolated steady state phase of accumulation is 5-6 μmole Ca^{2+} per gram membrane protein. The finding that the burst size is approximately twice the maximum level of phosphorylation is consistent with 2 to 1 stoichiometry of Ca^{2+} accumulated to ATP hydrolysed reported by other laboratories.

B. Ca^{2+} accumulation by microsomes prepared from young and old rat hearts: In vitro Ca^{2+} accumulating activity of sarcoplasmic microsomes prepared from young and old rat myocardium has been measured by a millipore filtration technique. Accumulation by microsomes from both age groups show a second order or cooperative dependence on Ca^{2+} ion concentration with substrate inhibition appearing at concentrations above 2 μM. Ca^{2+} uptake by microsomes prepared from old animals is significantly less ($p < .01$) than that of microsomes from young animals at all concentrations of Ca^{2+}. The mean level of a mitochondrial marker enzyme is the same in both populations, consequently the difference is not attributable to an increased mitochondrial contaminant in microsomes prepared from old animals. The differences in Ca^{2+} sequestering activity are consistent with the observation that old rat hearts show a prolonged contraction duration and suggest a possible biochemical mechanism for the physiologic change.

C. Cyclic AMP-dependent phosphorylation of neuronal microsomes: Rapid quenching has been used to follow the kinetics of cyclic AMP-dependent phosphorylation of an intrinsic membrane protein in bovine brain synaptosomes. The phosphorylation reaction has been implicated as an obligatory step in a catecholamine-induced process leading to an altered electrical state of the neuron. The induced electrical changes occur rapidly, attaining equilibrium within 500-600 milliseconds. The measured half-life of the phosphorylation reaction at less than saturating concentration of ATP is 250 milliseconds indicating that the reaction is fast enough to participate as an intermediate step in the electrophysiologic mechanism.

D. Kinetics of $(Na^+ + K^+)$-dependent ATPase: The transient state kinetic behavior of electric organ $(Na^+ + K^+)$-ATPase has been studied in the absence of added K^+. The behavior conforms to a simple scheme in which the acid - stable phosphorylated intermediate (E~P) breaks down directly to inorganic phosphate and free enzyme: $E + ATP \rightleftharpoons E \cdot ATP \xrightarrow{Na^+} E\sim P \rightleftharpoons E + P_i$. The presence of a second ATP binding site is inferred from double reciprocal plots of ATP concentration vs. P_i liberation which show downward curvature. The level of phosphorylation is twice that found in the presence of K^+ where sites are equally divided between the acid-stable (E~P) and acid-labile (E·P) intermediate states. Rate constants for the $(Na^+ + K^+)$ dependent partial reactions were evaluated for the following scheme using a nonlinear least squares data fitting program:

$$E + ATP \underset{(1)}{\rightleftharpoons} E.ATP \overset{Na^+}{\underset{(2)}{\rightleftharpoons}} E\sim P \overset{K^+}{\underset{(3)}{\rightleftharpoons}} E\cdot P \underset{(4)}{\rightleftharpoons} E + P_i.$$

Rate constants for ATP binding (k_1) and phosphorylation (k_2) evaluated from data obtained in the absence of K^+ provide an equally good fit to the partial reactions measured in the presence of Na^+ and K^+. In addition the rate constants corresponding to ATP dissociation (k_{-1}) and P_i release (k_4) increase with ATP concentration whereas the rate constant governing the formation of E~P from E·P (k_{-3}) decreases. A large shift in the ratio of acid-stable to acid labile sites is prevented by the inverse changes in k_{-3} and k_4. A 'half-of-sites' mechanism provides a possible explanation for this behavior as well as for the discrepancy between the number of ATP binding sites evaluated from kinetic and equilibrium binding measurements.

Significance to Bio-medical Research and to the Program of the Institute: A first step in understanding how the isometric performance of the heart changes with age is to understand the elementary steps of the transport processes which are involved in regulation of that activity.

Proposed Course of the Project: Transient state kinetic studies of Ca^{2+} uptake using colorimetric and isotope filtration methods will be continued to identify the specific steps in the transport mechanism which are affected by aging. The partial reactions of ATP hydrolysis coupled to transport will also be investigated to further characterize the steps involved in the active transport of cellular cations.

Publications:

Froehlich, J. P. and Taylor, E. W.: Transient state kinetic effects of calcium ion on sarcoplasmic reticulum adenosine triphosphatase. J. Biol. Chem., in press.

Froehlich, J. P., Albers, R. W., Koval, G. J., Goebel, R. and Berman, M.: Evidence for a new intermediate state in the mechanism of $(Na^+ + K^+)$-dependent adenosine triphosphatase. J. Biol. Chem. 251: 2186-2189, 1976.

Froehlich, J. P., Sullivan, J. V. and Berger, R. L.: A chemical quenching apparatus for studying rapid reactions. Anal. Biochem., in press.

NIA ANNUAL REPORT
July 1, 1975 through June 30, 1976
Gerontology Research Center
Laboratory of Behavioral Sciences

In a number of experiments over the last several years this laboratory has shown that cognitive performance declines with age. This is true whether one measures learning, memory or problem solving ability. Taken by itself, however, this finding is unsatisfying and nihilistic. It is unsatisfying because one would like to know more about the mechanisms underlying such an effect, and it is nihilistic because one would like to know about alternative strategies that he might develop to enable older subjects to cope effectively irrespective of such declines. It should be noted that these two issues of mechanism and coping strategy are not exclusive. It is only after one begins to understand mechanism that he can expect to develop techniques for enabling subjects to cope effectively with their deficits.

Psychologists evaluate cognitive function in two, seemingly different ways. One group of investigators studies such behavior by means of paper-and-pencil tests; the other group uses experimental interventions. The paper-and-pencil or psychometric techniques are likely to yield descriptive data about steady-state performance. Such analyses are valuable because they provide reliable characterizations of behavior, and because they permit the use of very powerful multivariate statistical methods to classify behavior. Experimental interventions also are valuable because they enable one to control a number of variables precisely, and utilize much more powerful means to identify specific mechanisms. While these two strategies clearly are complementary, they often have been contrasted as though they were mutually exclusive. In the past year a study was carried out in this laboratory which used multivariate statistical methods to evaluate the results of experimental studies of cognitive function. The subjects were 96 healthy, educated men ranging in age from 20 to 80 years. The analyses identified four factors of cognitive function: 1) speed of information processing; 2) secondary memory; 3) primary information processing efficiency; and 4) attention. These factors correlated with age: .36, -.40, -.32 and -.40 respectively. In the case of each factor, better performance was associated with younger age. Additional analyses within this study showed that the same factors emerged for each of three age groups; i.e., the structure of intellectual function does not differ with age, only the ability to perform declines.

One can find a number of references in the biographies of great scientists which indicate that so-called daydreaming played major roles in the creative processes which underlay their important discoveries. It is surprising, therefore, how little scientific research there is on daydreaming as a mechanism of problem solving behavior. This issue seems especially salient in the study of aging since it has been suggested that the age-related decline in daydreaming is a causal factor in the decline in creativity which supposedly occurs as people grow older. Several studies in this laboratory have been directed at analyzing the age-related factors in daydreaming. The finding that daydreaming occurs in men of all ages but

that the frequency of daydreaming declines with age has now been replicated. Two daydreaming factors, which were identified in previous studies of young adults as neurotic-anxious and obsessional-emotional daydreaming, have now been shown to be present at all ages. The neurotic-anxious factor was typified by mindwandering, boredom, distractibility, and absorption in daydreaming. The obsessional-emotional factor included daydreaming contents of guilt, fear of failure, heroism, and hostility, and also frightened reactions to and hallucinatory-vividness in daydreams. The present study not only showed that these factors were present at all ages, but also that they declined with age so that the daydreams of older subjects are lower in neurotic-anxious and obsessional-emotional content. Sexual daydreams are present in all men (over the age range of 25 to 91 years). Such daydreams also decline with age, and their frequency is related to the amount of sexual activity of the subject. In still another study it was shown that for both men and women, daydreaming about the past is unrelated to age. Daydreams about the past, present, or future were equivalent at all ages for both sexes.

As indicated above, it not only is important to identify the behavioral mechanisms underlying age-related declines in intellectual function; it also is important to develop strategies for enabling elderly subjects to cope with such effects. Research in this laboratory in previous years suggested that one of the mechanisms underlying age-changes in memory is a breakdown in the ability of elderly subjects to encode information uniquely so that it is no longer possible for them to retrieve the information on demand even though the information is stored in memory. Studies completed this year have shown that it is possible to train elderly subjects to utilize a mnemonic technique for encoding newly learned information so that it becomes possible for them to retrieve such data. The technique requires the subject to prepare an imaginary trip to a series of well-known places (e.g., rooms in his home). Then he associates each of the items to be learned with stopping places on the imagined trip. After such a mnemomic is learned it can be used in naturally-occurring situations, e.g., during grocery shopping.

In addition to the human studies described above, this laboratory also supports an active animal program designed to isolate still further some of the behavioral mechanisms associated with aging. Earlier data from this laboratory showed that low dietary protein increased longevity in each of two inbred strains of mice -- a naturally long-lived strain (C57), and a naturally short-lived strain (A/J) -- and in the F_1 cross. Current data indicate that within each of the six combinations of these three genetic groups and the two diets, longevity was inversely related to growth rate; i.e., the slower the growth, the longer the life. These data support the interpretation that the increased longevity of mice fed low-protein diets is a result of the reduction in growth rate induced by the diet. Other data from this project show that mice fed low-protein diets are significantly less active than controls. This finding clarifies the contradictory results which prevail in the literature: The reasons that some investigators have reported increases in activity during protein deprivation are that they were studying younger animals which were still developing, and that the observation period was too short. Another study in this laboratory

has helped to clarify the relative roles of genetic and environmental factors in behavior. Ethanol preference changes in mice whether the ethanol is palatable (as in the C57 strain) or unpalatable (as in the A/J strain). The preference declines from age 5 months to age 14 months, but increases thereafter. These effects not only appear in the parental strains but in the F_1 and F_2 hybrids as well. Genetic analyses of these data show that the degree of genetic determination for ethanol preference was smallest at senescence. These data add further credence to the hypothesis that genetic determinants of behavior decrease (and experiential determinants of behavior increase) with age.

Studies of long-term effects of operant conditioning of heart rate in the monkey are being carried out in this laboratory. During the first phase of this project heart rates and blood pressures were monitored during 18 hour periods, five days/week over a five week period. The results showed that for each of six animals heart rates and blood pressures were somewhat lower during the hours when the laboratory was quiet (8 P.M. to 8 A.M.). However, the differences between the quiet hours and the other times when there was activity in the laboratory (6 P.M. to 8 P.M. or 8 A.M. to noon) was modest in terms of <u>levels</u> of heart rate or blood pressure. The remarkable differences among these time periods were the changes in the <u>correlations</u> between heart rate and blood pressure. During the active hours the correlations were consistently high (.66 to .68) but during the quiet hours the correlations were consistently low (.10 to .15). These data suggest strongly that the extent of integration of cardiovascular function depends importantly upon the circumstances under which the animal is studied. When the animal is quiet and undisturbed, its heart rate and blood pressure are not strongly coupled. However, under conditions such as those one would ordinarily encounter in a laboratory, there is a high degree of coupling. Thus, usual laboratory studies may yield a distorted (or at least biased) picture of the interaction among the component responses of the cardiovascular system.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00061-14 LBS

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Behavioral Genetics and Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Charles L. Goodrick Research Psychologist LBS GRC NIA
OTHERS: None

COOPERATING UNITS (if any)

Baltimore City Hospitals

LAB/BRANCH

Laboratory of Behavioral Sciences Section

SECTION

Learning and Problem Solving Section

INSTITUTE AND LOCATION

NIA, GRC, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.10	.50	.60

SUMMARY OF WORK (200 words or less - underline keywords)

The major purpose of this project is to determine behavioral differences throughout the lifespan for populations of mice which differ in genetic structure, and which also differ in longevity. The present topics of interest concern the relations of growth rate, maximal body weight and longevity for mice fed low levels of dietary protein and for groups fed adequate dietary protein, in addition to studies of aging and voluntary wheel exercise, and ethanol preference.

PHS-6040
(1-75)

Project Description:

Objectives. The principal objectives of this project are (1) to determine group differences for behavioral traits and longevity among inbred strains of mice; (2) to determine heritability (degree of genetic determination vs. degree of environmental determination), mode of inheritance (e.g., over-dominant, dominant, intermediate, or recessive), and number of segregating units (gene blocks) controlling a particular trait (e.g., longevity); and (3) to examine relative behavioral differences among mouse strains as aging progresses. Other objectives include determining the influence of diet (e.g., protein available) on behavioral traits, growth, and longevity; and identifying single-gene influences upon behavioral traits, growth, and longevity.

Methods Employed: Inbred mice (C57BL/6J and A/J) of a high degree of homozygosity are maintained under uniform environmental conditions. The animals are tested behaviorally during one period of their life span, viz, when mature, mature-old, or aged. Old age is determined as the 50% mortality point for groups maintained throughout their life span. Statistically reliable techniques have been developed to determine behaviors relevant to natural selection such as exploration, general activity level, emotionality, simple or complex problem solving ability and taste preference. The use of segregating F_2 hybrid groups allows an estimate of the mode of inheritance, e.g., dominant or intermediate, and the number of gene blocks or segregating units controlling behavioral traits or life span. For studies in which protein intake is varied for groups of inbred and hybrid mice, isocaloric synthetic diets are used. Deprived animals receive 4% casein in their diets, whereas the control group receives a 26% casein diet Numerous kinds of mutant mice are maintained on the C57BL/6J background at the Jackson Memorial Laboratories; Bar Harbor, Maine. Our work has concentrated on the albino, beige, yellow and obese mutations.

Major Findings:

A. A large portion of the world population has a maintenance diet which is characterized by very low levels of dietary protein. The effects of this diet are a reduction of body weight and a concomitant slowing of growth rate. We have been testing the hypothesis that the effect of protein malnourishment may increase longevity due to slowing of the rate of growth using inbred and hybrid mice as subjects. In addition, studies of behavioral differences as a function of dietary protein and stage of development are in progress.

This year the study of longevity was completed using C57BL/6J mice, A/J mice, and hybrid F_1 mice (C57BL/6J X A/J), with 100 mice per group. Fifty mice of each group were fed low dietary protein (4%), and 50 mice of each group were fed moderate dietary protein (26%). The effect of low dietary protein was to increase the mean longevity for all groups, compared to the mean longevity for groups fed moderate dietary protein. Within all six groups, negative correlations were obtained between longevity and growth rate (peak body weight divided by growth duration). Five of the six correlations were significant at the level of .01. This is strong evidence for the hypothesis that the mechanism for the increment in longevity for groups fed low dietary protein is a slow growth rate.

B. One characteristic finding has been that protein malnourished animals are more active than animals fed adequate protein. These results have normally been obtained in other laboratories for animals given brief tests at an early developmental stage. Our results, for animals tested at maturity (42 malnourished and 42 controls) were that protein malnourished mice were significantly less active than controls.

C. A study of ethanol preference of mice has found that age is an important determination of ethanol preference. Inbred mice were studied for which ethanol solutions are highly aversive (A/J) or highly palatable C57BL/6J), in addition to hybrid F_1 and F_2 groups. In general, preference for ethanol decreased greatly with increasing age from 5 mo. old to 14 mo. old, and increased after 14 mo. of age through senescence. This age related difference in ethanol preference may be related to a decrement with age in responsiveness to novel stimuli, along with an increment in sensory thresholds with increasing age. The inbred strains were more similar in advanced old age with respect to ethanol preference, heritability of ethanol preference was reduced at senescence, and the number of gene loci which control ethanol preference were reduced after maturity. These findings support the hypothesis that genetic determinants of behavior decreases with age in fully developed animals.

Significance to Bio-Medical Research and Program for the Institute: The study of the genetics of behavior and longevity allows an assessment of: (1) the mode of inheritance (i.e., dominant, intermediate, etc.) for the factor studied (2) the relative importance of hereditary and environmental factors; and (3) the number of genes or gene blocks which control the factor studied. Lack of adequate dietary protein is a condition which affects a large proportion of the world populations. This project attempts to determine the effect of diet (such as different proportions of protein in the total diet) during particular stages of the life span upon behavior and longevity for animal populations which differ in genetic constitution. Studies of single gene mutant animals are of importance because they allow the assessment of the importance of a specific genetic locus for physiological or behavioral factors.

Proposed Course of Project: Further inbred strains and F_1 hybrid groups are being studied to determine the generality of mode of inheritance of behavioral factors. Cross-sectional and longitudinal studies of mouse behavior will continue with various mouse strains. The longevity of inbred and hybrid groups are also being determined. Experiments with low and normal protein diets should determine: (1) the relationships between growth rate and longevity, (2) the effect of low, normal, or high protein diets upon behavior at maturity after access to these diets during various stages of development, and (3) the effects of a diet of low, normal or high protein at the time of measurement upon behavior.

Publications:

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[illegible] PROJECT NUMBER (Do NOT use this space)	[illegible], EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-AG 00062-03 LBS

PERIOD COVERED July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Daydreaming and Aging: Normative and Experimental

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Leonard M. Giambra,	Senior Staff Fellow	LBS	GRC	NIA
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COOPERATING UNITS (if any) Baltimore City Hospitals; Morgan State University; Towson State College; College of Notre Dame of Maryland; Human Performance Section, Clinical Physiology Branch

LAB/BRANCH Laboratory of Behavioral Sciences

SECTION Learning and Problem Solving Section

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.55	.50	.05

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this work is to determine the parameters of spontaneous-thought-intrusions (daydreaming) and related mental activity such as insight and intuition. This is to be done through the use of retrospective questionnaire and direct experimental manipulation. Topics of present interest are: a) the influence of age and sex, b) the relation of sexual daydreaming and sexual activity, and c) the temporal orientation of daydreams.

PHS-6040 (11-75)

[redacted] goals are (1) to determine the incidence and content of daydreaming in specific subpopulations (e.g., young, middle aged, elderly) in various socio-economic classes, various races, etc; (2) to attempt to relate these differences in daydreaming to any underlying mechanisms such as physiological state, education, cultural values and beliefs, differential daily experiences, and (3) to investigate experimentally variables which normative studies have indicated may be potent determiners of daydreaming.

Methods Employed: The normative aspects of daydreaming are determined through the use of structured self-report. Each participant completes a 21 item biographical questionnaire and a 344 item Imaginal Processes Inventory (IPI) which has both specific and general items concerning daydreams, night dreams, fantasy, etc. To date approximately 1800 individuals from a wide variety of subpopulations have completed the IPI and bio-questionnaire. There are 28 scales in the IPI. Each item has five choices which are points on a continuum implying frequency or quantity. The choices were assigned values of 0, 1, 2, 3, or 4.

Major Findings:

I. Adult male daydreaming across the life span: A replication, further analyses, and tentative norms.

Since the earlier study was based on a sample of modest size, 378 adult males 17 to 91 years of age, the previously reported outcomes needed additional support as to their stability and reliability. To this end a replication sample of 218 men age 17-91 years of age was also asked to give retrospective reports of their daydreaming.

Since the effects of many demographic variables on daydreaming characteristics are essentially unknown the replication sample was chosen from the same population as the original sample. The men 24 years and older were, as in the original sample, participants in the Baltimore Longitudinal Study at the Gerontology Research Center of the National Institute on Aging. The 17 to 23 year old men were students, at large state universities--Miami of Ohio in the original sample and Towson State of Maryland in this replication sample. For the non-students there were 10% with less than one year of college, 10% with one to three years of college, 25% with four years of college, and 55% with more than four years of college education. Less than 10% could be described as lower or lower-middle class. Less than 5% were non-whites. The Towson State students were paid volunteers while all others were unpaid volunteers. The age distribution for the replication sample was: 17-23 (n=50), 24-34 (n=20), 35-44 (n=21), 45-54 (n=28), 55-64 (n=44), 65-74 (n=31), 75-91 (n=24). Except for the much smaller 17-23 year old age group, each age group was similar in size to that in the original sample.

Were the previously reported findings replicated? A review of the statistical comparisons between the original and the replication samples must lead to an affirmative reply. That is, the outcome of the replication sample vis-a-vis daydreaming and related mental activity across the life span seems to be equivalent to that of the original sample within the bounds of sampling error.

Based upon the larger combined sample one can conclude with some certainty that for middle and upper-middle class white males: (a) acceptance of daydreaming prevails in all age groups; (b) daydreaming frequency/intensity/absorption decreases steadily with increasing age with the 17-34 and post-65 year old intervals showing the most rapid decreases; (c) at all levels of frequency/intensity/absorption decreases with increasing age group and older, higher tendency daydreamers showed levels of frequency/intensity/absorption which in most instances exceeded that of younger, lower tendency daydreamers (d) about 8% of men in any of the age groups revealed that some of the time they had difficulty distinguishing their daydreams from reality; (e) mindwandering, boredom, distractibility, and need for external stimulation all decreased with increasing age group while mentation rate and both interpersonal and impersonal-mechanical curiosity remained essentially unchanged from 17-91; (f) except for a somewhat increased future orientation for 17-23 year olds and a somewhat reduced past orientation for 30-34 and 50-54 year olds daydreaming about the past, present or future was equally likely both across and within age groups; (g) both positive and negative emotional reactions in daydreaming decreased with age so that such reactions are minimal in the post-75 year old group; (h) higher frequency daydreamers showed a higher tendency to have both positive and negative emotional reactions, (i) for equally frequent daydreamers, visual imagery decreased very little with age, while auditory imagery showed a marked tendency to be largely missing with post-55 year olds; (j) for equally frequent daydreamers, daydreams which relate to solutions to problems and puzzles that the men come up against at work and at home were the most likely kind of daydream for all age groups except for the 17-23 year olds; (k) daydreams about sexual activity and love were most dominant for the 17-23 year olds then most rapidly decreased in likelihood with increasing age groups so that they largely disappeared after the 75th year; (l) daydreams about bizarre-improbable events, achievement, hostility, heroism, fear of failure, and guilt also became less and less likely with increasing age until all contents except problem solving content became rare.

The preceding enumeration should do much to dispel some of the long-standing but inaccurate notions about daydreaming across the lifespan and in the elderly in particular. Daydreaming does not (1) increase with old age, (2) focus in old age primarily on the past and (3) concentrate on the weird, outlandish, and improbable at any age. Daydreaming seems to be useful and of a problem solving nature focusing on our day-to-day life and puzzles which express our "current concerns."

Insight into the stable dimensions of daydreaming and related mental activity was provided by the results of the factor analyses of the original, replication, and combined samples. These factor analyses, when compared with the results of other factor analyses which included personality trait measures, provide a most convincing overview of the fundamental dimensions of retrospectively reported daydreaming and how these fundamental dimensions are age related. Singer and Antrobus reported the results of their factor analyses on a young college sample of men and women. Comparisons which follow are with the factors of the combined sample.

On Factor 1 there loaded highly the contents of guilt, fear of failure, heroism, hostility, achievement, and sexual activity as well as frightened reactions to daydreams, hallucinatory-vividness and absorption in daydreams. Singer and Antrobus reported a nearly identical factor which they labeled "Obsessional-Emotional Daydreaming." They also reported no direct link in this factor to any of the pathological scales of their personality inventories. Nonetheless, they chose to characterize this factor as representative of the pathological neurotic syndrome of tortured inner thoughts and fantasies full of guilt and self-doubt. Acceptance of daydreaming and age have unstable negative loadings on this factor. The absence of any loading for the positive reactions to daydreaming scale surely supports the negatively valued interpretation of this factor. Obsessional-emotional daydreaming seems to be a major dimension of daydreaming and also seems to have a weak, unstable but inverse relation to increasing age.

Factor 2 shows that impersonal-mechanical curiosity and problem solving daydreams loaded stably and highly on it while future in daydreams, acceptance of daydreams, achievement oriented daydreams, and positive reactions to daydreaming also loaded highly but unstably on it. This factor clearly reflects the analytic, productive problem solution dimension of daydreaming which is not age related. Singer and Antrobus did not report a similar factor.

Factor 3 found mindwandering, boredom, distractibility, daydreaming frequency, absorption in daydreaming and sexual daydreams loading highly positive while age loaded highly negative. Singer and Antrobus reported a factor on which these characteristics also load highly and on which personality characteristics such as neuroticism and emotional instability also loaded. They characterize this dimension of daydreaming as "Neurotic-Anxious Absorption in Daydreaming." This dimension thus seems to represent the non-productive semi-debilitating aspect of daydreaming with clear evidence that this decreases with increasing age.

Factor 4 was bi-polar with a present time orientation to the daydreams at one pole and bizarre-improbable daydreams at the other. This factor did not appear for Singer and Antrobus although these two aspects of daydreaming appeared at opposite poles on several of their other more inclusive factors. The mundane-improbable dimension of daydreaming is represented here and is not age related.

Singer and Antrobus reported a factor which they labeled "Social Extroversion" which reflects an active outgoingness and on which the need for external stimulation and self revelation scales loaded highly positive. These two scales comprised the highest stable loading on Factor 5. Age loaded highly at the other pole of this factor indicating the inverse relationship between social extroversion and age.

Factor 6 found interpersonal curiosity, past events in daydreams, self revelation and future time setting in daydreams all highly and stably loading on it. Interpersonal curiosity and future orientation of daydreams were prominent

on a Singer-Antrobus factor which was labeled "Controlled Thoughtfulness" since the Guilford-Zimmerman Thoughtfulness and Restraint scales were the two highest loadings on this factor. Factor 6 however, seems at best to be tenuous support for such a dimension operating in this sample. Perhaps Factor 6 represents a personal-subjective dimension of daydreaming which is the complement of the impersonal-objective dimension of Factor 2.

Factor 7 had all but one of the first seven daydreaming scales which loaded on a factor that Singer and Antrobus labeled "Positive-Vivid Daydreaming." This factor, which is not age related, indicates a dimension which involves a positive enjoyment of the vivid imaginal aspects of daydreaming.

In summary there are four orthogonal dimensions of daydreaming unrelated to age: "Impersonal-Mechanical Problem Oriented Daydreaming" (Factor 2), "Mundaneness of Daydreaming Content" (Factor 4), "Self-Other Personal Orientation in Daydreaming: (Factor 6), and "Positive-Vivid Daydreaming" (Factor 7). Two orthogonal factors were inversely related to age: "Neurotic-Anxious Absorption in Daydreaming" (Factor 3) and "Social Extroversion" (Factor 5). In addition, Factor 1, "Obsessional-Emotional Daydreaming," was found to be unstably and inversely related to age. What has emerged is an indication that with increasing age a steadily reducing part is played by the less desirable and more maladaptive dimensions of daydreaming while the positive, pleasant, and productive dimensions remain unchanged.

The extent to which these findings indicate cohort differences as opposed to developmental changes remains unanswerable until a longitudinal study is completed. The Baltimore Longitudinal Study Group on which the daydreaming information was collected will allow for the sampling of the same information from the periodically returning participants.

II. Sexual daydreams and quantitative aspects of sexual activity: Some relations for males across adulthood

Previous investigators have reported a direct relationship between sexual responses on a thematic apperception test and weekly orgasmic frequency. These investigators interpreted their findings as indicating a direct relation between sexual drive and sexual fantasy. Also reported among women was that sexual fantasies and daydreams (a) were common during intercourse, (b) increased sexual desire and pleasure, and (c) were unrelated to neurotic daydreams and problems of relationship, competence, or enjoyment of sexual activities. The present study analyzes retrospective reports of sexual daydreaming and their relationship to three behavioral aspects of the sexual history of adult males.

A total of 277 males from 24 to 91 years of age participated. These men were part of the Baltimore Longitudinal Study. The men were divided into six age groups as follows: 24-34 (n = 23), 35-44 (n = 27), 45-54 (n = 56), 55-64 (n = 83), 65-74 (n = 48), and 75-91 (n = 40). The men were predominately middle or upper-middle class whites with 88% having some college and 46% with a masters or doctoral degree; less than 10% were either black or other than

middle class, and virtually none were both black and non-middle class. All were volunteers in a program which asks participants to return to the Gerontology Research Center for re-testing every 12 to 24 months.

Aspects of daydreaming and related mental activity were measured from participant responses to the Imaginal Processes Inventory. Sexual Daydreams are measured by Scale 19 of the Imaginal Processes Inventory.

The three behavioral aspects of sexual history used in this study, were obtained in individual interviews conducted by Clyde E. Martin. Participants were each asked to contribute to a study of marital and sexual activity by means of a one-to-one interview. Cooperation was excellent with only 2.1% refusing to be interviewed. Each interview followed a predetermined sequence of questions. Most interviews were completed in a single session of two hours.

The unweighted arithmetic mean of the intercorrelations among the items of the Sexual Daydreaming Scale was .52 which yields a reliability of .93. Scale 28 of the Imaginal Processes Inventory indicates Self-Revelation, i.e., the degree to which each participant was willing to reveal himself honestly. A group mean of 21.3 was obtained for Scale 28. A mean of this magnitude can be interpreted as a willingness to reveal oneself since a scale value of 12 may be taken to indicate that it was "usually not true" that a participant was willing to reveal himself to others and a 24 may be taken to indicate that it was "usually true." Supporting this confidence in the honest self revelation by the participants was the generally high values of Scale 4 (Acceptance of Daydreaming). The group mean for this scale was 32.1 indicating that it was much more than "usually true" that the participants accepted daydreaming as natural and common and therefore revelation should be minimally affected by social desirability factors.

No firm statement based on separately acquired data can be made regarding the reliability and validity of the information relating to past sexual conduct since data collection was confined to a single interview. However, Kinsey, Pomeroy, Martin, and Gebhard whose interviewing methods largely paralleled those used in the present inquiry, found a satisfactory level of correspondence between husband and wife reports of common sexual activity and between two interviews with the same subjects spaced nearly three years apart. Unfortunately, their data did not include the behavioral measures used in this study. There is reason to believe that the sexual information obtained and used herein are of excellent quality since (a) all interviews were conducted by a highly experienced interviewer, (b) numerous aids to memory were incorporated into the question schedule, and (c) the behaviors involved are generally viewed by males as significant life events which tend to be well-recalled.

Number of coital partners and sexual daydreams. This sexual activity measure is the number of coital partners that the participant had had prior to the sexual behavior interview. Since any extended interval between this interview and completion of the Imaginal Processes Inventory would probably result in an erroneous value for this sexual activity indicator relative to Sexual

Daydreaming Scale values, analyses were restricted to a separation not exceeding two years. Four groups were used: age 24-44 and one to five coital partners (n = 12), age 24-44 and seven or more coital partners (n = 21), age 45-64 and one to five partners (n = 10), and ages 45-64 and seven or more partners (n = 11). A 2 X 2 harmonic mean unequal cell frequency analysis of variance using the Sexual Daydreaming Scale value as the dependent measure was performed with significant effects obtained for age group, and number of coital partners, but not for their interaction. The effect of age group was expected since the correlation of the scale and age was -.46 (n = 216, $p < .01$) and a like-valued correlation had been reported previously. The correlation of number of coital partners and age was -.04. For both age groups those with seven or more coital partners had more frequent and vivid sexual daydreams as indicated by greater means on Scale 19. Furthermore, although not significant the decrease with age in Sexual Daydreaming Scale values for participants with seven or more coital partners was less than half that of those with one to five coital partners.

Early marital coitus and sexual daydreams. Early marital coitus was defined as the "customary frequency of coitus during the first year or two of marriage." Three levels were considered--less than twice per week, two to less than four times per week, and four to less than eight times per week. The correlation with age at completion of the Imaginal Processes Inventory was zero, while the correlation with Sexual Daydreams was .12. An analysis of variance (harmonic mean unequal cell frequency) with four age groups and three frequency levels yielded significant effects due to age and to early coital frequency, but not due to their interaction. All coital frequency categories showed a decrease in Sexual Daydreams with increasing age. Furthermore, except for the 65-74 age group, the greater the customary frequency of coitus during the first year or two of marriage the greater the mean Sexual Daydreams Scale values. Also the decrease in mean sexual Daydreams Scale values with increasing age was at a greater rate with greater customary frequency of coitus so that for the 75-91 age group mean Sexual Daydreams Scale values were about the same for each coital frequency. For all participants (n = 500) interviewed the correlation between early marital coital frequency and number of coital partners was .22 indicating that these two measures appear to assess somewhat different aspects of sexual activity.

Quantity of sexual activity between ages 20 and 40 and sexual daydreams. In the interview concerning sexual and marital history, close attention was given to determining the presence or absence of coitus, masturbation, nocturnal emission, and homosexual activity in the participant's life history, and when present, their ages at onset, duration in time, and frequencies relative to age and marital condition. Since it has been shown that these behaviors account for nearly all male ejaculatory performance their frequencies of occurrence taken together constitute a measure of level of sexual functioning which is essentially unobtainable by other methods. In the present analysis, the frequencies of these activities were summed to a single number to indicated the quantity of ejaculatory activity reported for the period between 20 and 40 years of age. This number was then divided by 1040 weeks which allowed participants to be classified according to their mean weekly frequency for the twenty year interval. Such a measure of frequency of sexual activity

represents a long-term indication of earlier sexual vigor for those over 40 years of age and thus is restricted to that age period. The correlation of Sexual Daydreams and actual quantity of sexual activity between ages 20 and 40 was .24 and the partial correlation holding age constant was .13 while the correlation of sexual activity and age was -.17.

A 3 X 3 harmonic mean unequal cell frequency analysis of variance yielded marginally significant effects due to age and to sexual activity, but not for their interaction. The smaller the quantity of sexual activity the smaller the mean Sexual Daydreams Scale Values at every age group; however, the effect was minimal for the 65 and over age groups. The rate of decrease of mean Sexual Daydreams Scale values appeared to be greatest with the sexually most active. For all participants interviewed, the correlation between quantity of sexual activity during ages 20 to 40 and: (a) number of coital partners was .17, (b) early marital coitus was .70. This seems to indicate that quantity of sexual activity ages 20 to 40 reflected a somewhat different aspect of sexual behavior than number of coital partners but aspects of sexual behavior similar to that reflected in early marital coitus frequency.

The relation between sexual vigor as indicated by the quantitative measures of sexual activity and the frequency and intensity of sexual daydreams was found to be direct at all age levels except in the eldest where sexual daydreams rarely occurred. Since level of sexual vigor and intensity/frequency of sexual daydreams directly covaried, support was given to a current concerns position regarding the origin of daydreaming. The position that sexual drive and sexual fantasy directly covary cannot be ruled out, since increased sexual drive may be also inferred from the quantitative measures of sexual activity used. It has been argued, however, that many studies of the relation between different drives and fantasy production have obtained fantasy content of a particular type in the absence of the appropriate corresponding drive and that such fantasy production can be accounted for by the individuals' current concerns as set in motion by the accessible, appropriate incentives operating at that time. Of course, it cannot be ruled out that reported sexual activity and sexual daydreams may covary because of individual tendencies to over- or under-emphasize sexual material. Yet, might not any over- or under-emphasis merely reflect the real level of concern of an individual, especially those showing over-emphasis?

III. Daydreaming about the past: The time setting of spontaneous-thought-intrusions

It is commonly held that the elderly person's mental life is dominated by thoughts about the past. This belief forms the basic postulate of a psychiatric theory of positive personality adjustment in the elderly. The theory characterizes thinking about the past as spontaneous and unselective and also seen in adolescence and middle-age but with less intensity and frequency.

A daydream is defined as "...a shift of attention away from an ongoing physical or mental task or from a perceptual response to external stimulation toward some internal stimulus." Thus a daydream is a spontaneous intrusion of a thought or series of thoughts into the awareness of the person and hence ought to reflect any tendency for the aging person to think more about the past. The determination of the daydreaming characteristics, especially temporal orientation, was made by asking a sample of men and women 17 to 92 years of age to complete The Imaginal Processes Inventory. The Imaginal Processes Inventory has 28 scales of which Scale 10 (present-Oriented Daydreams), Scale 11 (Future in Daydreams), and Scale 12 (Past in Daydreams) deal with the temporal setting of daydreams. These scales have a potential range from zero to 48 with larger values indicating greater frequency/intensity/likelihood of that temporal setting.

The sample of participants in this study consisted mostly of white, well-educated people who may be classified as members of the middle and upper-middle class. The 17-23 year olds were college students. The 24-92 year old males were participants in the Baltimore Longitudinal Study at the Gerontology Research Center of the National Institute on Aging. The 24-92 year old females consisted of residents from the Baltimore metropolitan area and friends, wives, sisters, daughters, and co-workers of the males in the Baltimore Longitudinal Study. All elderly persons lived in non-institutional settings and were well-functioning members of the community. The number of individuals in each age group was as follows: 17-23 (male = 240, female = 362), 24-29 (m = 15, f = 52), 30-34 (m = 22, f = 45), 35-39 (m = 19, f = 45), 35-39 (m = 19, f= 48), 40 - 44 (m = 14, f = 19), 45-49 (m = 26, f = 29), 50-54 (m = 23, f = 24), 55-59) (m = 38, f = 18), 60-64 (m = 37, f = 24), 65-69 (m = 20, f = 28), 70-74 (m = 19, f = 13), 75-92 (m = 29, f = 11).

Correlations among the temporal orientation scales and each scale with age are presented in Table 1. It is clear from this table that past orientation in daydreams did <u>not</u> increase with age. Present oriented daydreams showed a slight tendency to increase with age with females but not with males. Future events in daydreams showed a modest negative correlation with age. An examination of the mean values of each age group on the future daydreaming scale (#11) indicated that this modest correlation was primarily the result of the decrease in future daydreaming during the 17-29 age interval and no decrease thereafter, except for the females which showed another decrease in the 75-92 year old group. No significant correlations among the temporal orientations of daydreams was observed for males while significant but very modest negative correlations between the present orientation and each of the other orientations was observed for females.

The failure to find any positive correlation between the past in daydreams (Scale 12) and age does not rule out the possibility that the past gains strength relative to the future or the present with increased age. To examine this possibility each participant was given a past-present and a past-future difference score. The past-present difference score was obtained by subtracting Scale 10 (Present-Oriented Daydreams) from Scale 12 (Past in Daydreams); the past-future difference score was obtained by subtracting Scale 11 (Future in Daydreams) from Scale 12. For these difference scores a positive value was greater.

[illegible] shaped relationship for both sexes with the 30 to 60 year [illegible] being the minimum--where the present most exceeds the past--and the ear[illegible] and later years being the maximum with the exception of the 70-74 year old females. Table 1 shows that the past-present difference-age correlation was essentially zero for both sexes. There was a modest tendency for the past to gain in strength relative to the future with increasing age. The future clearly exceeded the past orientation for the 17-23 year olds while the inverse was true for the 65-69 year olds. The 75-92 year old females provided the most extreme case where the past exceeded the future. Table 1 shows that the past-future difference-age correlation was significantly positive for both sexes but very modest in magnitude.

The above statistics provide scant support for the hypothesis that a past orientation increases with age. However, it is possible that, while the past orientation does not increase with age, with increasing age the more distant past is represented more than the recent past. Three items of Scale 12 are directly related to this issue. Item 163 asks each person about daydreams which involved events which happened more than one year ago. The aged failed to show any greater affirmative response to this item than any other age group except the 30 to 39 year olds. Item 296 asks about daydreams involving people and places with which the person was familiar when he or she was younger. Again the aged failed to show any greater tendency to respond affirmatively than any other age group. Item 273 asks about events from childhood. In this item there was a slight tendency for the aged to respond more affirmatively relative to the other age groups--the correlation of this item with age was .13 for females and .08 for males.

Table 1

Correlations Involving Temporal Orientations of Daydreaming

Correlation Between	Males[a]	Females[b]
Past and Age	-.005	.039[c]
Present and Age	.048	.137***
Future and Age	-.261***	-.247***
Past-Present Difference and Age	-.031	-.051
Past-Future Difference and Age	.185***	.195***
Past and Present	-.084	-.168***
Past and Future	.040	-.057
Present and Future	-.085	-.150***

a. Sample sizes range from 503 to 513
b. Sample sizes range from 675 to 701
c. No male-female differences in correlation are statistically significant ($p > .05$).

*** $p < .001$

In summary, daydreaming about the past did not show any tendency to increase with age. On an absolute basis the mean scale values for daydreaming about

the past exceeded the mean scale values for daydreaming about present and daydreaming about the future for the 65-69 year olds and the 75-92 year olds but not for the 70-74 year olds. Furthermore, little support was evident that the elderly daydreamed about the more distant past more often than the younger members of this sample. When past in daydreams mean scale values were compared with future in daydreams mean scale values across the life span the future exceeded the past except as just stated. However, the difference in the future and past scale values showed a tendency to decrease with age with a modest positive correlation between this difference and age. Thus one must conclude that any tendency for the elderly to daydream about the past is slight when the magnitudes of their temporal scale means are compared among themselves. When temporal scale differences are considered, the past daydreaming scale means approach and sometimes exceed that of the future and the present.

One implication of this study is that there is nothing about advanced age per se which makes thought about the past dominant. What is needed is a much more refined and intensive look at life events and circumstances to ascertain those which cause one temporal orientation to dominate. For example, perhaps the expectation of death or the advent of such events as marriage, empty nest, and anticipated retirement might lead to increased daydreams involving one particular temporal orientation.

IV. Male-Female differences in daydreaming across the life span

A sample of 392 females age 17-92 years of age have completed the Imaginal Processes Inventory and the Biographical questionnaire. Analyses are now being undertaken which will allow for detailed determination of male-female similarities and differences across the life span. Some preliminary results pertaining to temporal orientation of daydreaming have been determined and are reported above in III.

V. Black-white differences in daydreaming

Blacks 17 to 45 years of age have completed the Imaginal Processes Inventory and the Biographical Questionnaire and data analyses are progressing.

VI. The relationship of temperament and daydreaming in males across life span.

The members of the Longitudinal Study Group have completed the Guilford-Zimmerman Temperament Scales and correlational and factor analytic methods are now being utilized to determine the extent to which aspects of daydreaming and temperament are related.

Significance to Bio-Medical Research and Program of the Institute: The study of daydreaming is fundamentally a study of thought processes. In order to understand fully the thought processes of man, the total spectrum of those processes needs to be examined. In addition, it is important to know how this wide spectrum is affected by aging. Thus the study of daydreaming in

adults, along with other variables, such as differences in age [illegible] status, attitudes, etc., may help us understand the fundamental processes which underlie all these behaviors.

Proposed Course of Project:

1. Intraindividual age changes in daydreaming will continue to be studied.
2. The relationship of biological, social, and personality characteristics to daydreaming will continue to be explored.
3. Experimental studies on the time course of daydreaming will be initiated.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00063-09 LBS

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Learned Modification of Visceral Function in Animals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. T. Engel,	Chief, Lab. of Behavioral Sciences	GRC, NIA
OTHER:	J. A. Joseph	Staff Fellow	LBS, GRC, NIA

COOPERATING UNITS (if any)

Baltimore City Hospitals

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Psychophysiology

INSTITUTE AND LOCATION

NIA, GRC, Baltimore City Hospitals, Baltimore, MD 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.00	.50	2.50

SUMMARY OF WORK (200 words or less - underline keywords)

It is the long-term purpose of this project to study the psychophysiological mechanisms of learned control of heart rate in cardiovascular function in monkeys. The topics of present interest are the changes in cardiovascular function which occur during chronic exposures to operant cardiac conditioning.

PHS-6040
(11-75)

To determine the physiological and behavioral mechanisms associated with conditioned control of cardiovascular functions in the rhesus monkey.

Methods Employed:

This project is designed to study long-term effects of operant conditioning of heart rate. Subjects are four rhesus monkeys. Animals participate in a tri-phasic study. Phase I is five weeks duration. During this phase heart rate (HR) and blood pressure (BP) were monitored continuously for 18 hours (6 PM to noon the following day), 5 days/week. Phase II was a training phase during which animals 1 and 2 were trained to slow HR and animals 3 and 4 were trained to speed HR. Phase III is similar to Phase I except that on Monday, Wednesday and Friday animals 1 and 2 are required to maintain relatively slow HRs and animals 3 and 4 are required to maintain relatively fast HRs for the 18 hour period. If the animals fail to perform successfully, then the contingent training schedules are automatically initiated for 1024 sec. Phase III is still operative.

Major Findings:

A. Phase I

Only the data during this phase have been analyzed. Furthermore, for this phase only, the data for the four animals have been supplemented with data from two additional animals. Data from the 18 hour session are broken down into 128 sec segments -- in 18 hours there are 510 such segments. In this section we will report the results of analyses over the 18 hour time period (510 data points); over the time period 6:00 PM to 8:00 PM (55 data points); over the time period 8:00 PM to 8:00 AM (339 data points); and over the time period 8:00 AM to noon (116 data points). The 6:00 PM to 8:00 PM period reflects adaptation from the time when staff leave the laboratory; the 8:00 PM to 8:00 AM period covers a time when the laboratory and building are quiet and the animals are undisturbed; the 8:00 AM to noon period is a time when there is a great deal of activity in the laboratory. Table 1 reports the average HR and BP for the animals during each of the time periods over the five weeks of the study. There is surprisingly little variation in the measures.

Table 1: Average HR and BP

	6 PM - 8 PM	8 PM - 8 AM	8 AM - Noon	6 PM - Noon
HR	139.1	130.9	140.7	133.8
SBP	120.2	119.3	124.9	120.7
DBP	76.2	75.0	78.8	76.0

The correlations between HR and BP are given in Table 2. One of the most

interesting aspects of these data are the consistently low correlations which exist between HR and BP during the 8:00 PM to 8:00 AM period. Although under conditions of adaptation or during periods when there is considerable activity in the laboratory HR and BP are highly correlated, this is not so when the animals are at rest. Among other considerations about the integration of cardiovascular responses, these data indicate that the conditions during measurement strongly affect the nature of cardiovascular adjustments.

Table 2: Correlations of HR and BP

	6 PM - 8 PM	8 PM - 8 AM	8 AM - Noon	6 PM - Noon
HR X SBP	.66	.10	.78	.42
HR X DBP	.68	.15	.71	.39
SBP X DBP	.96	.93	.94	.94

B. Phase II and Phase III

As indicated above, these data have not yet been analyzed in any detail. All animals readily learned HR control during Phase II, and all animals have been in Phase III for at least six months.

Significance to Bio-medical Research and the Program of the Institute:

There is a widespread belief among biomedical investigators that "life-stresses" are important in the etiology of many diseases and in normal aging generally. Yet there are relatively few experimental data to justify this faith. One goal of this study is to determine whether chronic changes in cardiovascular function can be induced by learning.

Projected Course of Project:

A. Phase III of the present study will be continued.

B. New projects will be initiated: 1) to study the nature and mechanisms of cardiovascular-somatic integration; 2) to evaluate the changes in central neural dopamine receptor sensitivity in the rat as a function of age.

Publications:

Kristt, D.A.: Vasodepressor responses evoked from rat diencephalon: Effect of pre-stimulation blood pressure. Brain Research. 91: 156-160, 1975.

Engel, B.T., Gottlieb, S.H. and Hayhurst, V.F.: Tonic and phasic relationships between heart rate and somato-motor activity in monkeys. Psychophysiology, in press.

Thorne, P.R., Engel, B.T. and Holmblad, J.B.: An analysis of the error inherent in estimating heart rate from cardiotachometer records. Psychophysiology, in press.

METHOD OF PAYMENT (PLEASE INDICATE IF EXCHANGE) PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00064-15 LBS

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Problem Solving and Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIs:	David Arenberg	Chief, Learning & Problem Solving Section	LBS GRC	NIA
	Leonard M. Giambra	Senior Staff Fellow	LBS GRC	NIA

COOPERATING UNITS (if any)

Baltimore City Hospitals

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Learning and Problem Solving Section

INSTITUTE AND LOCATION

NIA, GRC, at Baltimore City Hospitals

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.85	.70	1.15

SUMMARY OF WORK (200 words or less - underline keywords)

The primary purposes of this project on aging are to: (1) identify reasoning processes; (2) determine how these processes change with age; and (3) develop techniques for reducing age deficits in reasoning performance. Reasoning is studied using problem solving procedures including concept learning and concept identification.

Project Description:

Objectives: The general goals are to explore and identify reasoning processes in man, to determine in what ways these processes change with age, and to develop techniques for reducing age deficits in reasoning performance. In this project, reasoning is studied by using problem-solving procedures in which on-going solution behavior can be observed and quantified. Experiments are designed to answer such questions as: (1) Is effectiveness in acquiring relevant information affected by aging? (2) Is effectiveness in synthesizing available information affected by aging? (3) What kinds of solution strategies are used and in what ways are they related to age? (4) How does imposing a memory load affect solution strategies for young and old adults?

Methods Employed: Experiment XI is a concept study in which each subject solves a large number of problems. The literature in concept identification is based almost entirely on mean effects of subsamples in which each subject solves one or a few problems. It has not been established that the variables which affect the mean performance of groups would affect an individual in the same way. When a subject solves one or a few problems, his approach to reasoning can be characterized as unstable, variable, transient, and highly subject to chance occurrences. Solving many problems is expected to result in a steady state of performance. In that steady state, strategies can be more readily elicited and the effects of various independent variables on changes in strategy and on other performance measures can be studied. After solving 48 complete learning problems, and again after 96, 8 more problems are solved in which subjects "think out loud" throughout each problem. When individuals "think out loud" they are verbalizing all the thoughts about the concept problem that are in their conscious awareness. They are specifically asked: (a) to relate the reasons behind their concept classification of the stimulus instance before them; (b) to tell if they had seen the stimulus instance earlier in the problem and if they remember its concept classification; (c) to relate how they use the feedback information about the correct classification of the stimulus instance before them to help them learn the concept; and (d) for any mnemonics they use to remember any and all aspects of a problem during the problem. The protocols of these "thinking-out-loud" problems constitute the primary data of this study. They are used to construct individual models of how a subject solves complex concept problems with which he is highly practiced.

Major Findings: In Experiment XI, complete data for the basic 96 problems plus 16 "think aloud" problems have been collected for 14 of the 17 subjects who have been included in the study thus far. The subjects range in age from 12 to 96 years. Model building is in progress for one 63 year old and one 18 year old man. Although for the early problems the performance of the five subjects over 80 was inferior to the younger participants, even these oldest subjects showed a steady improvement with increasing experience. One 90 year old woman attained a final level of performance which was indistinguishable from that of younger subjects.

After the basic problems, the oldest subject, a 96 year old man, was given similar problems in which the memory demands were reduced. Under this condition his performance improved greatly and was similar to that of the young. This experimental manipulation demonstrated that his reasoning ability was intact, but his performance on complex reasoning problems with a substantial memory component was impaired by his severely limited memory.

Significance to Bio-Medical Research and the Program of the Institute: Reasoning is among the most prized behaviors of man and among the most elusive for experimental study. In this project, methods have been and will be developed to obtain quantifiable measures of step-by-step performance on reasoning problems. Some of these methods also provide patterns of response which represent strategies in solving such problems.

Measures are obtained in current experiments to study changes in reasoning processes with age. These studies, in addition to identifying basic reasoning processes, should indicate the pervasiveness of reasoning deficits with age, whether education and cognitive activity mitigate such deficits, and what techniques could be used to minimize decline in reasoning.

Proposed Course of Project: Model construction will continue in Experiment XI. An attempt will be made to convert some experimental concept problems to paper-and-pencil format to answer questions about the effects of feedback messages and of the compatability of linguistic and logical aspects of feedback messages.

Publications:

Giambra, L. M.: Altering the concept learner's desire for nonexemplars of the concept: The effect of nonexemplar initial instances. Journal of General Psychology, 1975, 93, 213-232.

PROJECT NUMBER (Do NOT use this space)	NOTICE OF ANNUAL RESEARCH PROJECT	PROJECT NUMBER
		Z01 AG 00065-16 LBS

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Verbal Learning and Age

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David Arenberg	Chief, Learning & Problem Solving Section	LBS GRC	NIA
OTHER:	Elizabeth A. Robertson-Tchabo	Visiting Fellow	LBS GRC	NIA
	Carol Hausman	Student	Univ. of Md.	
	Jack Botwinick	Professor	Washington Univ., St. Louis	

COOPERATING UNITS (if any)

Baltimore City Hospitals
University of Maryland
Washington University

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Learning & Problem Solving

INSTITUTE AND LOCATION

NIA, GRC, at Baltimore City Hospitals

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.05	1.70	2.35

SUMMARY OF WORK (200 words or less - underline keywords)

The primary purposes of this project on aging are to identify measures of verbal learning and memory which change with age, to specify psychological processes and their relationships with age, and to develop procedures for improving learning and memory performance in the elderly.

Project Description:

Objectives: Primary objectives are: (1) to identify which aspects of learning and memory change with age (and which do not); (2) to specify psychological processes underlying such age changes; (3) to identify health, nutrition, biochemical, and personality variables which are correlated with performance or with change in performance; and (4) to develop procedures to improve learning and memory performance in the elderly.

Methods Employed: Experiments I and II are both longitudinal studies of verbal learning; a serial-learning procedure is used in Experiment I, and a paired-associate procedure is used in Experiment II. In each study, time to respond is a variable; and each participant is randomly assigned to a fast, slow, or subject-paced condition.

Experiment XXVI is a short-term-memory study in which mixed lists of digits and letters are presented visually; and both recall and recognition tasks are used. The digits and letters are presented sequentially at a one second rate on contiguous screens. At the end of each list the subject is cued to answer one of three questions: (1) when the task is to recall the item which appeared on one of the screens, a question mark appears on that screen; (2) when the task is position recognition, an item (a digit or letter) appears on one of the screens, and the subject indicates whether that item had appeared on that screen; and (3) when the task is list recognition, an item appears on an additional screen, and the subject indicates whether that item had appeared anywhere in the list. A certainty rating is made for each response. The three tasks provide a comparison of two kinds of recognition under conditions which induce a recall strategy. It is assumed that the additional information required to respond successfully in the position-recognition task imposes greater retrieval demands than does the list-recognition task. On that basis, it is hypothesized that age differences will be smaller for list recognition than for position recognition. It is also hypothesized that the recall task will result in large age differences due to the substantial search aspects of retrieval required.

Experiment XXX is a study of the effectiveness of a mnemonic device on learning by the old. The procedure is to learn a "trip" through the learner's living quarters. Most mnemonics require substantial practice to overlearn the procedure in order to use it, and would probably not be suitable for the old. It is expected that because the locations are part of their everyday living they will be readily learned by old men and women.

Experiment XXXI is a new study including several measures of memory, response speed, and attention. The tasks include immediate free recall, delayed free recall, delayed recognition, digit span, dichotic listening, simple reaction time, and four choice-reaction-time tasks in which the cognitive load is varied. The subjects are all college educated and healthy. Multivariate analyses were carried out to identify psychological processes (factors) and to determine the relationship of the factor scores with age.

Experiment XXXII is a new study, in collaboration with the Clinical Physiology Branch, of the effects of propranolol on learning and information processing. Learning is measured by a multi-trial, free-recall procedure. In addition, simple reaction time is included along with four choice-reaction-time tasks in which the cognitive load is varied. Subjects will receive an injection of propranolol or saline (double blind--neither the subject nor the experimenter knows what is injected). Several hours before and two hours after the injection, all performance measures and concurrent heart rate recordings are obtained. Two aspects of this study are: (1) the effects of propranolol on performance in young and old men; and (2) the relationship among heart rate, cognitive load, and performance in young and old men.

Major Findings: Experiments I and II are longitudinal studies of verbal learning. Previous results showed not only that performance was related to age, but that change in performance was related to age; in each study, at both a fast and a slow pace, means of the oldest groups declined the most. This year, longitudinal data for subjects who learned at their own pace (they had as much time as they needed to respond to each item) were analyzed. For both the serial task (N=86) and for the paired-associate task (N=78), change in performance was related to age with the oldest men declining the most. The results were quite similar to the findings for the paced aspects of the two studies.

Experiment XXVI is an age study of recognition and recall memory. It was hypothesized that: (1) list recognition would be easier than position recognition; (2) recall would be more difficult than either recognition task; (3) age differences would be found in all tasks but the differences would be dependent upon the degree of search required, i.e., largest for recall and smallest for list recognition. On the basis of partial data (79 men aged 31 to 88), all hypotheses are supported. These data further confirm the idea that a major component of memory decline with age is search of stored information. It should be noted that in the final phase of the experimental session when subjects were most highly practiced, age differences in list recognition were virtually nil (the men over 80 performing as well as those under 40) whereas age differences were moderate for position recognition and substantial for recall.

Experiment XXX is a study of a classical mnemonic procedure which was selected for attributes judged to be particularly effective for the old. The results showed that this mnemonic can be learned readily by elderly subjects, and that it is very effective in facilitating recall. The procedure is usable in real-life situations such as remembering grocery lists.

Experiment XXXI is a study of cognitive processes involved in ten different tasks, and the relationships of the processes with age. Thirteen measures based on performance of 96 healthy, educated men on these tasks were included in several factor analyses to identify cognitive processes. The factors were identified as speed of information processing, secondary memory, attention, and primary processing efficiency. The correlations of these

factors with age were -.35, -.30, -.40, and -.15 respectively, with better performance on each factor associated with lower age. Furthermore, the same four factors were identified in each of three age groups (20-39, 49-59, 60-80). This indicates that the processes are stable over age, but the level of performance based on these processes declines.

Experiment XXXII is a study of propranolol, performance, and age. The experimental procedures and the instrumentation were developed, and data collection was initiated.

Significance to Bio-Medical Research and the Program of the Institute: Learning is more central to experimental psychology than any other behavior, and some of the most striking and consistently reported behavioral age differences in the gerontological literature have been found in verbal learning performance. The experiments in this project are designed to identify basic mechanisms of learning and retention and to measure differences and changes in these functions that occur with age. In addition, knowledge about experimental variables which affect age differences will be valuable in developing techniques for optimizing learning of the older person.

Proposed Course of Project: A dichotic listening study will be initiated to explore the findings from a dichotic study in progress which is showing that some elderly men cannot attend to a single pair of simultaneous inputs (a different digit to each ear). Young men (and many old men) can handle single pairs with no difficulty. In the new study, onsets of digit pairs will be separated to determine the minimal asynchrony an old subject (who cannot identify simultaneous pairs) requires to identify both inputs. This separation time is an index of switching time required to attend to two digits presented close together in time. In this new study, which is conceptually similar to Experiment VIII of Project Number Z01 AG 00066-15, the stimuli are meaningful words (digits) rather than tones, and the performance is based upon correct identification of the two stimuli rather than response time to the second tone. The study takes advantage of the fact that some elderly men cannot identify both digits when they are presented simultaneously, one to each ear. Switching time will be related to several aspects of the EEG and possibly to evoked potentials or contingent negative variation.

Current plans are to include in the Longitudinal Study the multiple measures from the cross-sectional study in which process factors were identified. Such a plan will provide measures of individual age changes in measures of those cognitive factors.

Publications:

Arenberg, D: The effects of input condition on free recall in young and old adults. Journal of Gerontology, in press.

Arenberg, D. and Robertson-Tchabo, E. A.: Learning and aging. In Birren, J. E. and Schaie, K. W. (Eds.): Handbook of the Psychology of Aging. New York, Van Nostrand, in press.

Botwinick, J. and Arenberg, D.: Disparate time spans in sequential studies of aging. Experimental Aging Research, in press.

Robertson-Tchabo, E. A.: Immediate free recall as a function of age, input modality, and presentation rate: Primary and secondary components. Journal of Gerontology, in press.

Robertson-Tchabo, E. A. and Arenberg, D.: Aging, learning, and problem solving. In Wolman, B. B. (Ed.): International Encyclopedia of Neurology, Psychiatry, Psychoanalysis, and Psychology. New York, Van Nostrand Reinhold, in press.

Robertson-Tchabo, E. A. and Arenberg, D.: Age differences in cognition in healthy, educated men: A factor analysis of experimental measures. Experimental Aging Research, in press.

Robertson-Tchabo, E. A., Hausman, C. P., and Arenberg, D.: A classical mnemonic for older learners: A trip that works. Educational Gerontology, in press.

PROJECT NUMBER (Do NOT use this space)	NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER
		Z01 AG 00066-15 LBS

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Perceptual Retention and Age

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David Arenberg	Chief, Learning and Problem Solving Section	LBS GRC NIA
OTHERS	Elizabeth A. Robertson-Tchabo	Visiting Fellow	LBS GRC NIA
	Walter W. Surwillo	Professor	Univ. of Louisville

COOPERATING UNITS (if any)
Baltimore City Hospitals
University of Louisville

LAB/BRANCH
Laboratory of Behavioral Sciences

SECTION
Learning and Problem Solving Section

INSTITUTE AND LOCATION
NIA, GRC, at Baltimore City Hospitals

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.45	.10	.35

SUMMARY OF WORK (200 words or less - underline keywords)

The primary purposes of this project on aging are: (1) to investigate perceptual retention and interference; (2) to determine under what conditions age differences in retention are affected by interference; and (3) to investigate processes of interference and perception. Current studies involve the psychological refractory period and non-verbal memory.

Project Description:

Objectives: One general objective is to investigate the effects of interference in perceptual retention and in perception: (1) to determine whether aging results in increased susceptibility to interference; (2) to explore conditions which affect age differences in interference; and (3) to develop procedures for testing mechanisms which may account for the empirical findings. Another objective is to study non-verbal memory and the conditions which improve such memory, especially for the old.

Experiment VIII is a study of age and the psychological refractory period. When two signals are presented close together in time and the task is to respond to the second, the reaction time increases as the interval between signals decreases (over the range of 500 to 50 msec). The additional time required is known as the psychological refractory period. One explanation is based on a model involving a single channel of limited capacity which processes information sequentially. When two signals occur successively at a fast rate, the system cannot attend to and process both. As a result, the second signal must be stored until the previous signal has been processed. It has been hypothesized that the refractory period (the delay) is related to the dominant alpha period of the EEG. If so, the refractory period should be longer for the old than young adults and should be related to the period of concurrent EEG alpha waves. A study has been planned to compare the refractory period of young and old adults, and to correlate refractory period with EEG alpha.

Major Findings: In Experiment VIII the apparatus was completed and data collection was initiated. No analyses have been made of preliminary data, but the effects of the interstimulus interval is evident in virtually every subject. Whether that effect is greater for the old (supporting the hypothesis that the refractory period increases with age) will not be known until more data are collected.

Significance to Bio-Medical Research and the Program of the Institute: The general idea that a person becomes more susceptible to interference as he grows older is well entrenched in gerontological thinking and is often used to "explain" age differences in performance. The evidence for this idea, however, is sparse and not consistent. It is the purpose of this project to explore the generality of the age-interference hypothesis for non-verbal memory and perception. It is important, both for theoretical and applied reasons, to identify those conditions which are especially interfering for the old. Furthermore, correlative studies of interference behavior and physiological variables, such as the alpha period of the EEG, should improve our understanding of the processes underlying the behavior.

Proposed Course of Project: If warranted by the results of Experiment VIII, study of the psychological refractory period will be pursued with concomitant cortical electrophysiological measures such as the EEG period, evoked potential, and contingent negative variation.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00067-09 LBS

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Learned Modification of Visceral Function in Man

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. T. Engel	Chief, Lab. of Behavioral Sciences	GRC, NIA
OTHER:	S. A. Rubin	Clinical Associate	LBS, GRC, NIA

COOPERATING UNITS (if any)

Baltimore City Hospitals

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Psychophysiology

INSTITUTE AND LOCATION

NIA, GRC, Baltimore City Hospitals, Baltimore, MD 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.40	1.40	2.00

SUMMARY OF WORK (200 words or less - underline keywords)

It is the long-term purpose of this project to study the clinical application of operant conditioning of visceral responses, so called biofeedback. The present interests are in patients with chronic, severe fecal incontinence, patients with cardiac arrhythmias and patients with high blood pressure.

PHS-6040
(1-75)

Project Description:

Objectives:

A. Learned control of blood pressure: 1) To develop a physiologic characterization of the cardiovascular response to learned blood pressure control; 2) To assess the efficacy of outpatient training of hypertensives learning blood pressure control; 3) To study the relationship of peripheral venous volume and tone to left ventricular filling as a function of age.

B. Learned control of rectal function: 1) To determine whether patients with irritable bowel syndrome (IBS) can be trained to control recto-motor responses; 2 To determine whether elderly patients with histories of chronic, severe fecal incontinence can be trained to regain control over anal sphincter function.

Methods Employed:

A. Learned control of blood pressure.

1. This project was discontinued because the technical requirements of the measurement procedures interfered too much with the performance.

2. Patients in this pilot project will take part in a triphasic study. Phase I is one month and will comprise a baseline period during which patients will take their blood pressures at home daily, and during which their physicians will take their pressures weekly. During Phase II and Phase III patients will be trained either to relax, or lower their pressures using a self-control method we have described earlier. During Phase III there will be a reversal; i.e., patients who were trained to relax now will be trained in self-control and patients trained in self-control now will be trained to relax. Each phase will comprise three months.

3. Normal, healthy subjects aged 20-35 (young) or 45-60 (middle aged) are being studied. Subjects are tested during four intervention procedures: a) bilateral thigh venous occlusion; b) blood volume expansion; c) inhalation of amyl nitrite; d) sub-lingual administration of nitroglycerin. Cardiovascular measurements include: 1) Heart rate by electrocardiography; 2) blood pressure by sphygmomanometry; 3) left ventricular end diastolic volume by cardio-ultrasound; 4) left ventricular stroke volume by cardio-ultrasound; 5) forearm blood flow by strain gage plethysmography; forearm venous pressure through a venous catheter (this is the only invasive measurement technique in the study).

B. Learned control of rectal function

1. This project was discontinued.

2. Efforts are underway to initiate this project as a collaborative study with one of the Veteran's Administration Geriatric Research and Education Centers (VA-GREC).

Major Findings:

A. Learned control of blood pressure

1. Project discontinued.

2. There are too few data to permit any assessment of findings.

3. Since this study still is ongoing, data analysis are limited; the findings reported below are tentative, and are not based on statistical analyses.

a) Mechanical changes in total blood volume (by venous occlusion or saline infusion) produced modest changes in cardiovascular function. Under these conditions the left ventricular function changes can be adequately explained by the Frank-Starling rule. Pressure-volume relationships in the limb can be explained in terms of the passive, mechanical properties of the veins.

b) Pharmacologically induced changes in peripheral volume (nitroglycerine or amyl-nitrite) produced larger changes in cardiovascular resonses. These responses can, in part, be explained by the Frank-Starling rule; however, neuro-motor relexes (e.g., baroreceptor reflexes) also seem to contribute importantly to cardiovascular regulation. In general, the changes in venous compliance which have been observed seem to act to preserve ventricular filling (i.e., end-diastolic volume).

c) There may be an age relationship between peripheral venous regulation and central volume; however, there are too few data to warrant more than the suggestion that this may be so.

B. Learned control of ano-rectal function

1. Project was discontinued.

2. Over the last several years the Baltimore City Hospitals Gastrointestinal Unit has treated 40 patients with histories of chronic severe fecal incontinence. Conservatively, 70% of the patients were treated successfully (i.e., disappearance of incontinence or reduction of frequency of incontinence by at least 90% over follow-up periods ranging from four months to eight years). The age-range of patients treated was 6 years to 96 years. The findings strongly indicate that operant conditioning is a powerful and optimal therapy for such patients. These findings further seem to warrant efforts to attempt to treat elderly patients. For that reason efforts have been initiated to establish a cooperative project with a VA-GREC Unit.

Significance to Biomedical Research:

Behavioral treatment of medical disorders is a new development in medicine. This laboratory has contributed importantly to such development. Such therapy is very important since it is based on the rational application

of well-established behavioral principles, and since it optimizes the implementation of naturally occurring physiological responses. Such methods contrast sharply with pharmacological interventions which are inherently unnatural.

Proposed Future Course:

On-going projects will be continued. Every effort will be made to get the VA-GREC cooperative study underway. After 1 July 1976 a study will be initiated to evaluate the effectiveness of behavioral intervention in acutely ill patients on a cardiac care unit. Subjects of all ages will be included.

Publications:

Engel, B.T.: Clinical applications of "biofeedback" in the treatment of fecal incontinence and in the control of some cardiac arrhythmias. Tribuna Medica (Madrid). XII: No. 610 (p.36) and No. 611 (p. 36), 1975.

Roessler, R.R. and Engel, B.T.: The current status of the concepts of physiological response specificity and activation. Psychiatry in Medicine. 5: 359-366, 1974.

Weiss, T. and Engel, B.T.: Evaluation of an intra-cardiac limit of learned heart rate control. Psychophysiology. 12: 310-312, 1975.

Engel, B.T.: The Cardiac Arrhythmias. In Budzynski, T.H. (Ed.): Biofeedback: The research and application, in press.

Engel, B.T.: Operant conditioning of cardiovascular function: A behavioral analysis. In Rachman, S. (Ed.): Advances in Medical Psychology 1976. London, England, Pergamon Press, in press.

Engel, B.T.: The treatment of fecal incontinence by operant conditioning. In Ghista, D.N. (Ed.): Physiological Physics and Therapies. Madras, India, Marcel Dekker, Inc., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AG 00069-11 LBS

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Exercise, General Activity Level and Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Charles L. Goodrick Research Psychologist LBS GRC NIA
OTHERS: None

COOPERATING UNITS (if any)
Baltimore City Hospitals

LAB/BRANCH
Laboratory of Behavioral Sciences

SECTION
Learning and Problem Solving Section

INSTITUTE AND LOCATION
NIA, GRC, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.80	.40	.40

SUMMARY OF WORK (200 words or less - underline keywords)

The major goal of this project is to determine the effect of voluntary wheel exercise upon behavior for animals tested in lifespan developmental research, and in addition to consider the effect of voluntary wheel exercise upon longevity. Another goal of this research is to increase the period of vigorous activity during later stages in the lifespan of the rat or mouse.

PHS-6040
(1-75)

Project Description:

Objectives: The general objectives are: (1) to determine methods for increasing vigorous physical activity of lower animals during late stages in the life span (2) to examine behavioral and longevity differences among animals which differ in physical activity level, and (3) to determine the physiological mechanisms underlying differences in activity.

Methods Employed: Wistar rats or various strains of mice are placed in standard activity wheels and allowed access to free voluntary exercise. Hungry animals also may be rewarded with food for running. Other studies utilize inbred, hybrid, and mutant mice or species which differ in activity level due to different genetic constitutions (See Project Z01 AG 00061-14 LBS Behavioral genetics and aging).

Major Findings:

A. An ongoing investigation of voluntary wheel exercise and longevity is still in progress. In this study paired rats are maintained in cages with attached activity wheels (experimental groups) or in cages without access to activity wheels. The 40 males (Group 1, N=12; Group 2, N=28) and 40 females (Group 1, N=12; Groups 2, N=28) allowed access to activity wheels were started under these conditions when 45 days old. The two groups were started six months apart. Control pairs of 28 males and 32 females were also started at the age of 45 days. The only groups in which all rats have died are the male controls and male experimental group 1. For all other groups, some rats are still alive. However, it is possible to compare the two male groups with regard to longevity, and also to give the percentages of rats which have survived past 25 months of age for all groups. The mean longevity for the male control groups was 20.71 months and the mean longevity for male experimental Group 1 was 25.83 months. The difference in longevity for these two groups was highly statistically significant, t (38) = 4.92, $p < .01$. The percentage of rats which have survived past the age of 25 months are:

1. a. Male control, N = 28, 4%
   b. Male experimental, N=12, 58%
   c. Male experimental, N=28, 25%

2. a. Female control, N=32, 47%
   b. Female experimental, N=12, 67%
   c. Female experimental, N=28, 79%

For both male and female rats, the experimental groups have more long lived survivors than the control group. When all animals have died, the longevity data will be completely analyzed. In addition, analyses of body weight, metabolic rate, and voluntary wheel exercise will be completed.

B. Groups of normal and genetically obese mice were studied in an experiment during which dietary protein (low, 4%; moderate, 26%; and high, 48%) and cage environment (voluntary wheel exercise; standard cage) were varied. Each mouse was tested under one of the six conditions from the age

of 5 weeks to 29 weeks (a 24 week interval). Some of the major findings were: a. For normal mice, voluntary wheel exercise was a directly increasing function of dietary protein. Obese mice engaged in very little voluntary wheel exercise compared to normal mice after the first several weeks of the study; but for obese mice, voluntary wheel exercise was inversely related to dietary protein. b. Body weight increased directly with increased dietary protein, and mice allowed voluntary wheel exercise gained weight more slowly than mice in regular cages. c. The body weight increment of mice fed low protein diets was greater for obese mice than for normal mice, despite the fact that food intake was similar. A possible behavioral mechanism for the facilitation of weight increment was the reduction of fluid intake to less than 2 ml./day for obese mice in cages and less than 3 ml./day for obese mice in wheels. Fluid intake of normal mice is 4-5 ml./day. Normal mice fed low protein diets typically double their fluid intake.

C. Mature-young and senescent albino A/J, pigmented C57BL/6J inbred mice and their hybrid F_1s were tested under low or high illumination intensities to observe differences in self-selected wheel-running, bar-pressing, and light-dark durations behavior over time. The animals, individually isolated and tested 10 at a time in 12 homogeneous subgroups (N = 120), were always allowed ad lib food, water, and runwheel access. During the pre- and post-experimental phases, the mice were kept under standard LD 12:12 cyclic illumination; during the experimental phase, however, they were allowed to select their own light and dark schedules by pressing on either of two accessible bars, one light-contingent and the other dark-contingent.

Measures of general runwheel and bar-pressing activities, motivational aspects of illumination changes and intensity preferences, time series analyses of periodicities, power ratios, and significant other multiples were all obtained from the subjects during a total of three experimental phases of 10 days each.

Some of the results of this study were:

a. Aged mice were less active than young mice and differences in wheel activity of diverse genetic groups were less at senescence than at maturity. This basic finding has been obtained in our laboratory for other behavioral measures and may indicate a reduced importance of genetic factors as aging progresses.

b. Young mice spent much less time in the light than aged mice. This may be related to less activity of aged mice than young mice. Because mice are active in darkness and inactive during daylight, young mice may turn the lights off more than aged mice because of a requirement for darkness during activity.

c. The periodicity (cycle length, normally 24 hr.) of A/J mice was found to be greatly increased, compared to all other groups. Moreover, in general, wheelrunning periodicities of aged mice were less prominent (i.e., contained more random noise) than those of young mice, and aged mice showed a larger number of significant other periodic components besides the main than did

young mice. These age differences in periodicities indicated that the temporal relationships involved in activity patterns may be disrupted in advanced old age.

Significance to Bio-Medical Research and the Program of the Institute: One of the consistent findings of gerontological research is the decline in general activity level of old animals compared with young animals. It is important to determine whether quantity of activity (e.g., wheel activity) and/or quality of activity (e.g., increased exploration behavior or greater response variability) may be increased experimentally for old and senescent animals. It is also important to examine the role of heredity with respect to voluntary exercise througout the entire lifespan, and the effect of exercise upon behavioral decrements associated with advanced old age. The knowledge and utilization of factors which change base activity levels of aged animals may result in more productive later years for aged humans.

Proposed course of the project: The studies of rat wheel exercise will continue to determine the effects of voluntary exercise upon longevity for paired rats, and to determine the amount of voluntary exercise during advanced old age.

Studies of wheel exercise periodicity of young and aged mice will be continued. Periodicity patterns of mice will be examined throughout old age. Additional studies will determine the level of voluntary activity for young and aged mice which are allowed voluntary control of lighting conditions within the home environment.

Publications:

Goodrick, C.: Adaptation to novel environments by the rat: Effects of age, stimulus intensity, group testing, and temperature. Developmental Psychobiology, 1975, 8, 287-296.

Wax, T.: Age, strain, and illumination intensity effects on behavior: I. Self-selection of locomotor activity and of light-dark schedules. Journal of Comparative and Physiological Psychology, In Press.

Wax, T., & Goodrick, C.: Voluntary exposure to light by young and aged albino and pigmented inbred mice as a function of light intensity. Developmental Psychobiology, 1975, 8, 297-303.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AG 00068-14

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Operant Performance, Memory and Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Charles L. Goodrick, Research Psychologist LBS GRC NIA
OTHERS: None

COOPERATING UNITS (if any)
Baltimore City Hospitals

LAB/BRANCH
Laboratory of Behavioral Sciences

SECTION
Learning and Problem Solving Section

INSTITUTE AND LOCATION
NIA, GRC, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.20	.10	.10

SUMMARY OF WORK (200 words or less - underline keywords)

The major purpose of this project is to analyze complex maze learning of young and aged animals, and to determine techniques which act to improve learning ability. Another goal of this project is to determine age differences in operant performance for young and aged rats or mice, and to determine factors which may act to improve performance, and also improve retention of the learned responses.

PHS-6040
(1-75)

Project Descriptions:

Objectives: The general objectives of one phase of this project are: (1) to analyze complex maze learning of young and aged animals and (2) to determine variables which may act to enhance or retard maze learning ability. The objectives of the other phase of this project are: (1) to study age differences in motor performance during operant responding; and (2) to develop operant techniques which improve the retention of learned responses in aged animals.

Methods Employed: Operant conditioning performance and retention studies have used 2-bar test boxes in which hungry animals are trained to press one bar to obtain a food reward while the alternate bar remains neutral. By increasing the complexity of the task (using two bars rather than one), it is possible to make a finer analysis of performance and the retention process. We are studying performance and retention as a function of reward schedule, and we are particularly interested in the partial reinforcement effect. The retention of partially rewarded responses is vastly greater than responses continuously rewarded; and analysis of this phenomenon will provide information regarding the general retention process.

A complex 14-unit multiple-T maze also is utilized. This maze has been shown to be a highly reliable test of learning, and it has been used in many major studies of aging. Additional mazes of 6-units are being developed to study mastery of consecutive problems by young and aged rats. These mazes will be used to analyze aging effects in short-term and long-term memory and to determine aging effects in interference, both proactive and retroactive.

Major Findings: Data are currently being analyzed which will be reported in detail in next year's annual report.

Significance to Bio-Medical Research and the Program of the Institute: Learning and/or memory deficits represent a major problem among the aged human population. Major behavioral techniques to reduce performance deficits obtained for aged animals have been studied in our laboratory. This project may facilitate research with man by identifying optimal conditions for learning and for retention of learned responses.

Proposed Course of Project: Further studies are in progress to determine the nature of the partial reinforcement effect in relation to (a) time contingent vs. response contingent partial reinforcement, and (b) massed vs. distributed extinction trials. Other studies will examine age differences in operant performance as a function of response effortfulness. Maze studies will concentrate upon the effects of central nervous system stimulants on behavioral rigidity within the maze for old rats. We will also initiate preliminary studies of perceptual learning in humans to determine the generality of the massed practice effects found for aged rats.

Publications: None